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ORIGINAL ARTICLES

THE PROBLEMS OF ALLERGY IN THE AIR FORCE

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VERY little has been reported in recent years about the problems of allergy in the Air Force. Most of these studies were made during World War II.[1-3] In 1943 French and Halpin[2] organized a series of fifty-nine allergy clinics in the Fourth Corps Area. In these clinics, 32,046 patients with allergies of various kinds were studied. These included 6,842 cases of poison ivy dermatitis, 5,373 cases of seasonal hay fever, 3,831 cases of perennial allergic rhinitis, and 9,591 cases of asthma. In 1944 Hampton[3-4] reviewed the problem of allergy in the Army Air Force in a study of 1,898 allergic patients seen at the San Antonio Aviation Cadet Center, Lackland Air Force Base, Texas. He found that hay fever and asthma were the most important manifestations of allergy, asthma being the most important. These studies give evidence of the practical importance of allergy to the Air Force.

In 1953, nine years after the end of World War II, Fein[5] studied 400 cases of bronchial asthma among World War II veterans and showed that asthma was the most important allergy problem at that time.

How has this problem varied since World War II? This article is based on a study of 600 Air Force personnel seen in consultation in the allergy clinic at the United States Air Force Hospital, Lackland Air Force Base, Texas, during the year September, 1957, to September, 1958. The ages of the men and women ranged from 17 to 55, with 57 per cent under the age of 25 and 84 per cent under the age of 35 years. In addition to the 600 airmen, another 1,200 civilian dependents and retired personnel were examined in the clinic. These civilians were not included in this study.

Almost one-half of the patients were referred from outside the medical facilities of this Air Force Base. They came not only from Air Force bases throughout the United States but also from overseas. The major referring source was the hospital ear, nose, and throat service, and the remaining cases came from specialty clinics (Table I).

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TABLE I. NUMBER AND PERCENTAGE OF PATIENTS REFERRED AND PLACE OF REFERRAL

OFF BASE		ON BASE	
	194 (43%)	ENT	174 (38%)
		Other	91 (19%)
Total	194		265

The examination of each patient consisted of a complete history, physical examination, laboratory studies (including complete blood count), nasal smear for eosinophils, and sinus and chest roentgenograms. Pulmonary function evaluation was done as indicated.

The Air Force medical staff has become keenly aware of the need for early detection and treatment of allergic conditions. The careful screening of recruits is of utmost importance in reducing the incidence of allergy in the Air Force. Based on the experiences of investigators during World War II, definite physical standards have been recommended.[6] These standards provide for the rejection of recruits with severe nasal allergy, polyps, chronic sinusitis, bronchial asthma, and severe allergic dermatoses. The strictest requirements are applicable to aviation cadets, and a history of nasal obstruction or sinusitis may be an indication for rejection.[7] During this study period, we saw twenty-six aviation cadets in consultation and recommended disqualification for twenty-two, or 85 per cent, mostly because of respiratory allergy.

TABLE II. INCIDENCE OF ALLERGIC AND NONALLERGIC DIAGNOSIS

DIAGNOSIS	NUMBER	PER CENT
Rhinitis, allergic	295	46
Sinusitis	113	17
Asthma, bronchial	65	10
Rhinitis, vasomotor	45	7
Urticaria and angioedema	34	5
Undiagnosed conditions	23	4
Conjunctivitis, allergic	20	3
No disease found	17	3
Dermatitis, atopic	8	1
Nasal septal deformity	4	1
Rhinitis medicamentosa	3	
Bronchitis, nonallergic	2	
Dermatitis, contact	1	
Iritis	1	
Migraine	1	
Corneal ulcer	1	
Physical allergy	1	
Asthma, Yokohama	1	
Allergy, gastrointestinal	1	
Bronchitis, allergic	1	3
Meniere's syndrome	1	
Hyperhidrosis	1	
Purpura, idiopathic	1	
Erythema multiforme	1	
Retinitis pigmentosa	1	
Rhinitis, atopic	1	
Loeffler's syndrome	1	
Horton's headache	1	
Total	645	

Between August and December, 1958, allergy was responsible for 12 per cent of all medical discharges at this base. In this same period only 3 per cent were discharged because of cardiovascular disease. Hampton,[4] in 1944, pointed out that 8 per cent of all discharges at Lackland were due to allergy, mainly asthma. This change in diagnosis is striking. Notably low figures for asthma are seen at present, and the predominance of presenting symptoms in the nose and sinuses is noted (Table II). Table II also shows the diagnoses made in 645 patients and is very interesting as well as instructive. As expected, allergic rhinitis was diagnosed most frequently, there having been 295 cases (46 per cent). Evidence of paranasal sinusitis was present in 113 cases (18 per cent). Bronchial asthma was diagnosed sixty-five times (10 per cent). Interesting to note is the fact that vasomotor rhinitis was the most common nonallergic condition diagnosed.

The remaining diagnoses covered a variety of allergic and nonallergic conditions, which included such unusual chest diseases as Yokohama asthma first described by Redfearn and Karakawa[8] in 1953. It is seen that the cases studied, with the above changes, correspond quite closely with the cases studied by Hampton[3] at this hospital in 1945.

DISCUSSION

The need for maintaining large numbers of Air Force personnel in world-wide distribution and the necessity of attaining ever-greater altitudes and speeds have resulted in greater physical and psychological stresses. With this, allergy in the Air Force has taken on a new importance. Special problems have been created for the Air Force allergist. One of the problems results from the rotation of personnel, which has an effect on the diagnosis, course, and incidence of allergic diseases. To appreciate this factor, the allergist must be familiar with not only local but also regional atmospheric pollen conditions.[9] This hospital derived almost one-half of its patients from medical facilities of the Air Force throughout the United States and overseas.

It was noted that seasonal symptoms frequently became perennial as a result of transfer to areas with prolonged pollen seasons, particularly in the South, where in most places there is no severe freeze. This, no doubt, accounted for the predominance of perennial symptoms in our patients. Diagnostic difficulties were encountered in one-fifth of the patients, in whom seasonal patterns had been obscured by necessary travel or symptoms had just recently been triggered by the patients' entering areas with high pollen counts. Specific regional plants, such as the mesquite and mountain cedar trees of Texas, were sometimes responsible for changes in the course of the patient's disease.

The psychological stress of military life became manifest in several ways. Maladjustment was found prevalent mainly in the lower grades. Some airmen attempted to obtain medical discharges through the allergy clinic. The frequency with which nonallergic vasomotor rhinitis was seen further emphasized the role of emotional tension, frustration, and hostility in the production

of nasal symptoms.[10] The majority of patients with urticaria were referred to us because of the chronicity of symptoms and obscurity of cause. As expected, exacerbations of urticaria and angioedema were frequently related to emotional stress.

The most serious problem is the effect of allergic respiratory disease on flying personnel. The role of allergy in the production of barotitis and barosinusitis is well recognized.[11-12] With the advent of modern jet planes, the incidence of barotrauma has tripled in frequency.[13] For these reasons, even mild nasal allergies may ground a pilot. Bronchial asthma is a serious handicap in high-altitude flying.[14] Thirty-eight pilots on flying status underwent study in the clinic, chiefly because of respiratory allergy. One pilot was referred because of a contact dermatitis from his oxygen mask. Interestingly, the contact sensitivity could be attributed to iodochlorhydroxyquin ointment, which had been used in therapy and contaminated the entire face mask. Allergy treatment of flying personnel is difficult and particularly challenging. Therapeutic doses of antihistamines have been shown to reduce perceptual motor skills and cannot be used while on flying duty.[15] Personnel of the Air Force must, of necessity, be fit for worldwide duty, and transfer to specific or more suitable geographic areas for relief of allergy is beyond the realm of consideration. Hyposensitization of local patients was carried out at this facility. For referred patients, individual treatment kits were prescribed. This treatment, in traveling personnel, is often difficult and impossible.

SUMMARY

The worldwide distribution of Air Force personnel, with exposure to various regional pollens, climatic conditions, and other stressful physical conditions, influences the course and pattern of allergic diseases.

The detection of allergy at the recruiting level has caused the disqualification of applicants. Whereas in the past the primary symptom in recruits, as seen in 1944, was asthma, now the screening processes has helped to eliminate this. It is evident that the incidence of rhinitis and other upper respiratory tract diseases has increased. This group of diseases, rhinitis, and sinusitis need more observation and scrutiny.

As a result of this study, it becomes obvious that there is a definite need for training more physicians in the field of allergy in the Air Force to solve these problems.

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HYPOGAMMAGLOBULINEMIA WITH RESPIRATORY ALLERGY

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AGAMMAGLOBULINEMIA, first described by Bruton[1] in 1952 in an eight-year-old male child, is characterized by extreme susceptibility to bacterial infection, low gamma globulins, absence of circulating antibodies, immunologic unresponsiveness to antigenic stimulation, lack of isohemagglutinins, and clinical improvement with replacement gamma globulin therapy.

The studies on agammaglobulinemic patients by Janeway, Gitlin, and their co-workers[2-9] at Harvard University and by Good and his collaborators[10-15] at the University of Minnesota have clearly defined this entity and furthered our knowledge in the fields of immunology, hematology, and the chemistry and metabolism of gamma globulin.

Since immunochemical studies in agammaglobulinemic patients have shown the presence of at least some gamma globulin, the term "hypogammaglobulinemia," as proposed by Pearce,[16] has found increasing usage in the literature. However, Pappenheimer[17] doubts whether the small amounts of gamma globulin found by the immunochemical method have anything to do with antibody gamma globulin because of the universal failure to detect circulating antibody in these patients by the most sensitive tests. The two terms therefore will be used interchangeably.

Hypogammaglobulinemia may be classified as follows:

I. *Congenital.*—This type occurs in male children. It is an hereditary disease with a sex-linked Mendelian-recessive defect expressed only in boys.[3] The incidence is about one in 50,000 live births.[18] Symptoms develop soon after six months of age.

II. *Acquired.*—This type may be idiopathic (primary) or secondary.

A. *Idiopathic (primary) agammaglobulinemia* occurs in either sex and at any age. These patients handle infection well during the early years of life. This type may be a late expression of an inherited defect.

B. *Secondary agammaglobulinemia* is associated with disorders of the reticuloendothelial systems, such as multiple myeloma, chronic lymphatic leukemia, lymphosarcoma, and in association with rheumatoid arthritis,[13] scleroderma, dermatomyositis, and lupus erythematosis.[7]

III. *Physiological.*—This type is observed in the first few months after birth. It is due to a transient inadequacy of gamma globulin synthesis.

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Although there are approximately 80 reported cases of the congenital type and about half as many of the idiopathic acquired type, there are only a few reports of acquired primary hypogammaglobulinemia associated with clinical allergy.[19, 20, 21]

CASE REPORT

The patient, a 51-year-old white man, came to Phoenix, Arizona, from his farm in Minnesota in March, 1959, for climatic relief because of repeated severe febrile infections which involved the sinuses, middle ears, lungs, and eyes. He attributed these respiratory infections to a nasal and respiratory allergy. His nose was always blocked. He suffered from constant nasal purulent drainage complicated by severe sinus infections. He had developed nasal polyps. He constantly complained of redness of the eyes and photophobia.

He had been well until the age of 44. During each of the past seven winters he had been besieged with severe recurrent infections of the upper and lower respiratory systems. He recounted some fifteen episodes of pneumonia during the past seven years, each responding to large doses of penicillin and sulfonamides. In addition, he had had innumerable sinus and middle ear infections, as well as recurrent conjunctival infections.

He was not troubled with sneezing but his main complaint was nasal stuffiness throughout the entire year, aggravated during the fall and winter months. He felt best during the spring, summer, and early fall. His trouble always began in the late fall and lasted throughout the entire winter. In his home in Minnesota he had been bedridden the entire past winter because of repeated sinus, middle ear, eye, bronchial and lung infections. The only food to which he thought he was intolerant was frozen perch. Ingestion of this food caused nausea, vomiting, severe abdominal cramps, and diarrhea within two hours. He could eat other types of fish without any distress. He denied any sensitivity to drugs, pollen, poison oak or ivy, animals, tobacco or horse serum. Allergy skin tests (type not specified) performed one year previously in Minnesota revealed no positive reactions. His father had had bronchial asthma and died of cancer of the lung. His mother had had recurrent hives and died of cancer of the stomach. His youngest brother had bronchial asthma. His oldest brother had died of pneumonia at the age of 32 in the preantibiotic era. He stated that "we are a family of weak-lung people—both on my father's side and my uncle's (paternal) side."

In 1942 he had had a tonsillectomy and adenoidectomy. In 1954, because of continued cough and expectoration following an episode of pneumonia, he was subjected to bronchoscopy. No evidence of any bronchogenic tumor or abscess was found. Radiographic examination at that time revealed a marked increase in the bronchial markings compatible with severe bronchitis. In 1958 nasal examination by a rhinologist revealed a very severe type of allergic rhinitis. The postnasal chambers were filled with very large polyps, which were removed. The pathologic report on the specimen was as follows: Microscopic section reveals respiratory mucosa covering pedunculated polypoid structures with a loose edematous fibrous connective tissue and an infiltrate of leukocytes of which moderate numbers are eosinophils; there is no evidence of malignancy.

The history of repeated infections suggested the possibility of a gamma globulin deficiency. Serum electrophoresis performed in three different laboratories gave the values shown in Table I.

Isoagglutinins: His blood was of group A, Rh positive with no demonstrable anti-B isoagglutinins in the serum. The gamma globulin level by immunochemical quantitation was 100 mg. per cent which is considered a moderate gamma globulin deficiency. A Schick test gave a positive reaction. Skin test with second strength PPD gave a positive reaction. Cutaneous, intradermal, and passive transfer studies were performed with pollens, fungi, house dust, epidermal, miscellaneous (nonpollen) inhalants, foods, and bacterial vaccine. There were no immediate wheal and flare reactions elicited by any of the antigens. Passive transfer studies failed to reveal any evidence of skin sensitizing antibodies. However,

TABLE I

DETERMINATION	NORMAL	I	II	III
Total protein	6.2-7.8	4.92	5.07	6.0 Gm.
Albumin	3.6-4.7	3.74	4.18	73%
Alpha 1 globulin	0.2-0.4	0.24	0.15	5%
Alpha 2 globulin	0.5-0.7	0.47	0.38	12%
Beta globulin	0.8-1.1	0.40	0.34	8%
Gamma globulin	0.9-1.2	0.07	0.04	2%

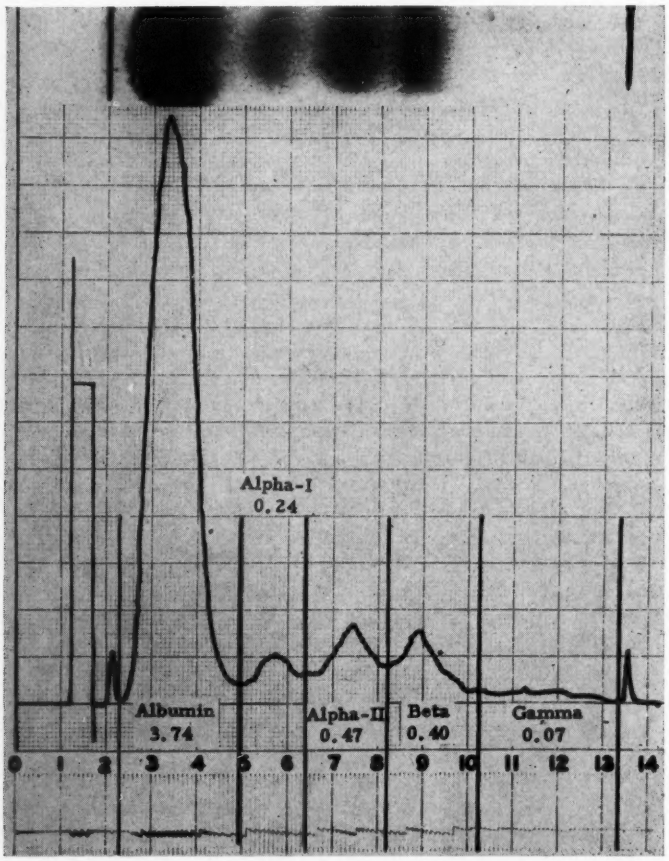

Fig. 1.—Serum electrophoretic pattern in hypogammaglobulinemia.

numerous delayed-type (24 and 48 hours) positive reactions were noted with the following antigens: dog and goat epithelium, kapok, orris, tobacco, wool, alternaria, hormodendrum, monilia *(Candida albicans)*, mucor, penicillium, gum karaya, barley, spinach, banana, cherry, pineapple, chocolate, and tea. There was no delayed type of reaction noted with any of the pollen allergens.

Physical examination except for the eyes was unremarkable. An ophthalmologist described the eye lesion localized to the most superficial layers of the corneas and confined entirely to the corneal epithelium. The lesion, as examined under the high power bio-microscopy and diagnostic staining, revealed punctate-like staining of the epithelium which increased in density toward the center of the cornea, the peripheral cornea being almost clear.

This had caused a reduction of vision to 20/100 in the right and left eyes. Local steroid treatment reduced the secondary inflammation of the bulbar conjunctiva, but in no way affected the primary lesion of the cornea.

His course during his stay in Phoenix from March to June, 1959, was uneventful. One mild febrile episode associated with a sinus infection was controlled by penicillin. His main complaint was the visual difficulty associated with the keratitis. A hemogram and urinalysis were normal. X-ray of the chest revealed no pulmonary infiltration or other abnormality. An electrocardiogram revealed no abnormality; other routine laboratory studies revealed no abnormalities.

DISCUSSION

The clinical diagnosis of hypogammaglobulinemia is suggested by a marked susceptibility to repeated episodes of bacterial infections. It is readily confirmed in the laboratory by electrophoresis which reveals an almost complete absence of gamma globulin from the serum and by demonstration of absence of isohemaglutinins. The Schick test will invariably be positive. Antigenic stimulation with triple typhoid and other potent vaccines will fail to demonstrate evidence of circulating antibodies. Ultimate proof may be obtained by immunochemical methods.

The patient with hypogammaglobulinemia, lacking circulating antibodies, is subject to serious bacterial and viral infections.[18] These are listed in Table II.

TABLE II. MAJOR COMPLICATIONS OF HYPOGAMMAGLOBULINEMIA

A. *Acute*	B. *Chronic*
1. Pneumonia—predominantly pneumococcal	1. Bronchiectasis—severe and extensive
2. Meningitis	2. Pansinusitis
3. Otitis media	3. Pyelonephritis
4. Hepatitis—serum	4. Moniliasis of mouth and nails
5. Pyelonephritis	5. Destruction of joints following septic arthritis
6. Septic arthritis	6. Brain damage due to plastic exudates
7. Septicemia (bacteremia)	7. Radiculoneuropathy from repeated meningitides
8. Acute infectious polyneuritis with permanent paralysis	8. Hypersplenism, peripheral adenopathy, hepatomegaly
9. Pyoderma	9. Sprue-like syndrome
10. Purulent conjunctivitis	10. Lung abscess

Chemistry and Metabolism of Gamma Globulin.—Serum proteins by electrophoresis separate into four fractions: albumin and three globulins, alpha, beta, and gamma. The gamma globulins can be further refined into gamma-1, a fast moving component, and gamma-2, a slow moving fraction, both of which contain antibodies. Sehon and co-workers[22, 23] have demonstrated that the skin sensitizing antibodies migrate electrophoretically as gamma-1 globulins, and the blocking antibodies are associated primarily with gamma-2 globulins. The molecular weight of the gamma globulins is approximately 156,000. Immunologically, the gamma globulins have common antigenic groups. Their clinical significance is their relation to antibody formation. Patients whose serum has little or no gamma globulin have a low resistance to infection.

The gamma globulins are synthesized by the plasma cell in the reticuloendothelial system and bone marrow.[4, 11] Upon their release from the plasma

cells they are distributed throughout the extracellular fluid in the body. The plasma concentration of gamma globulins is normally between 600 and 1,100 mg. per 100 ml., or 11 to 14 per cent of the total plasma proteins. About 3.5 per cent of the total gamma globulins in the body are turned over each day in normal persons. The catabolic half life of gamma globulins is about 20 days.[24] The normal infant begins to synthesize gamma globulin between four and twelve weeks of age.[25] Gamma globulin plasma concentrations within normal limits for the adult are reached between the ages of six months and seven years.[26] The lymphoid tissue of a newborn infant resembles that of the agammaglobulinemic patient. It shows poor corticle development in the lymph nodes with absent secondary germinal follicles and absent plasma cells. Any agent which causes an involution of lymphoid tissue, such as ACTH, cortisone[27] and its derivatives, x-rays, and nitrogen mustards,[28] will depress the synthesis of specific gamma globulin.

Hypogammaglobulinemia and Delayed Type of Allergy.—The relationship between hypogammaglobulinemia and the delayed type of allergy has been studied by Porter[29, 30, 31] and by Good and his co-workers.[11, 14, 15]

It is generally recognized that there are two distinct types of hypersensitivity: (1) the immediate type of reaction associated with the presence of circulating antibodies, and (2) a delayed type of reaction associated with cellular antibodies.

Within the category of immediate hypersensitivity are (1) the anaphylactic type comprising anaphylactic shock, Arthus reaction, and serum sickness, and (2) those of atopy which are associated with skin sensitizing but not precipitating or anaphylactic antibodies and include hay fever, extrinsic asthma, urticaria, angioedema, and gastrointestinal allergy. The delayed type of reactions are associated with cellular antibodies and include contact dermatitis and the tuberculin type of allergy.

The studies of a patient with hypogammaglobulinemia and respiratory allergy corroborates the theory that there is a dichotomy in the mechanism of hypersensitivity. The most sensitive tests available fail to detect the presence of any circulating antibody in the agammaglobulinemic patient.[17] Yet uniquely the mechanisms for development of the delayed type of hypersensitivity remains firmly intact in the agammaglobulinemic patient.[11] Thus, Porter[29, 30, 31] and Good and co-workers[11, 14] in their studies on hypogammaglobulinemic patients have been able to demonstrate the development of a delayed type of allergy to diphtheria toxoid, horse gamma globulin, Ascaris, polysaccharide antigen and to a hapten, 2,4 dinitrofluorobenzene (DNFB). These investigators have been able to transfer the delayed allergy induced in the hypogammaglobulinemic patient to immunologically normal persons by using viable leukocytes from the peripheral blood.

Dienes[32] was the first to demonstrate that when a protein is injected into the skin, the first response is the appearance of a delayed type of reaction. Pappenheimer and collaborators[17] believe that the injection of antigen into an

animal induces synthesis of two types of structures with configurations complementary to determinate groupings on the antigen molecule. One of these antigen binding sites is fixed to cells and is responsible for the reactivity associated with the delayed hypersensitive state. The other is antibody gamma globulin. That the formation of the factor responsible for delayed sensitivity is under separate genetic control from gamma globulin synthesis is shown by the ease with which patients with congenital agammaglobulinemia can be sensitized to antigens against which they cannot produce conventional antibody. In both cases antigen is probably required as a template to direct the folding of polypeptide chains to complementary configurations as postulated by Pauling.[33] Pappenheimer and his collaborators further propose that each of these two antigen-induced complementary structures is related to the other and represent successive steps in antibody synthesis. They suggest that the primary antigen-induced complementary configuration is located at or near the surface of cells and is the specific factor which accounts for the inflammatory reactions characteristic of the delayed hypersensitive state.[34] Secondary stimulation of sensitized animals with small doses of antigen may provoke a delayed progressive inflammatory reaction, or larger doses of antigen may result in desensitization. In either case, specific interaction of antigen with the complementary binding sites of certain sensitized cells result in capture of antigen by the cells and its retention at the site of globulin synthesis.

Relationship of Keratitis and Delayed Type of Allergy.—Is there a relationship between the corneal damage (keratitis) in this patient and the hypogammaglobulinemia or the manifestations of a delayed type of allergy? Raffel[35] who has studied the cornea in relation to delayed reactivity following sensitization of animals with egg albumin mixed with tubercle bacillary wax observed striking injury to the cornea. This was also noted by earlier investigators who studied the cornea in relation to delayed reactivity to the streptococcus,[36] the pneumococcus,[37] and the tubercle bacillus.[38] The same investigators observed no untoward reactions in the corneas of animals having the immediate type of reactivity induced by soluble protein antigens, including the nucleoprotein of the pneumococcus, the tubercle bacillus, or egg albumin.

That the hypogammaglobulinemia per se was not responsible for the corneal damage (keratitis) is suggested by the failure of adequate replacement with human gamma globulin to alter favorably the keratitis.

This would indicate that whereas replacement with gamma globulin may have a beneficial effect on the agammaglobulinemic patient's resistance to bacterial infection, it does not influence the effects of bacterial type of delayed hypersensitivity. It might also indicate that factors other than bacteria, that is, spores of fungi, other inhalants, and possibly even foods, all of which are capable of giving rise to delayed type of skin tests in this patient with agammaglobulinemia, may give rise to the manifestations of allergy induced by the delayed type of hypersensitivity alone.

Delayed reactions, particularly with intradermal tests, have been noted by many allergists. The clinical significance of the delayed type of skin test, however, has been a controversial subject. Thus, Fineberg[39] is of the opinion that in

atopic manifestations of delayed types of reaction are rarely of clinical significance. Black[40] believes that the delayed type of skin reaction after the scratch test has much significance, whereas after the intradermal test it is probably due to nonspecific irritants. Kern,[41] Cooke,[42] and Vaughn,[43] however, regard delayed reactions as important.

The agammaglobulinemic state is characterized by the absence of circulating antibody and the retention of the capacity for delayed type of allergy. In such a patient with clinical allergy, therefore, the demonstration of the delayed type of skin reactions should be correlated with the clinical symptoms and given the same significance as the immediate wheal and flare type of reaction in routine skin testing in the normal patient. The presence of a delayed type of response in routine skin testing by the intradermal method in allergic individuals with normal gamma globulin should not be ignored. The delayed type of skin test bears the same relation to the presence of cellular allergy as the immediate wheal and flare type to the presence of humoral antibody.

Therapy of Agammaglobulinemia.—In the presence of an acute episode of a specific bacterial infection, antibiotics are the first choice of therapy. Gamma globulin, however, is very effective in the prevention of severe recurrent bacterial infections in patients with hypogammaglobulinemia. Gross, Gitlin, and Janeway[24b] recommend the injection of 150 mg. of gamma globulin per kilogram or about 0.45 ml. of solution per pound of body weight for the prevention of bacterial infections in patients with agammaglobulinemia. The initial dose should be double this recommended dose. Injections should be repeated every four weeks thereafter to maintain a serum level above 150 mg. per 100 ml.

SUMMARY

Hypogammaglobulinemia is characterized by susceptibility to recurrent infection, low gamma globulin, absence of circulating antibodies and isohemagglutinins, and immunologic unresponsiveness to antigenic stimulation. There are two main types, the congenital, a sex-linked recessive trait expressed in male children, and the acquired, occurring in either sex at any age. A case of acquired hypogammaglobulinemia is described in a 51-year-old man with respiratory allergy and keratitis. The absence of skin sensitizing antibodies in the hypogammaglobulinemic patient was demonstrated by P-K passive transfer studies. The capacity to develop delayed-type allergy was demonstrated by delayed-type skin reactions, not only to tuberculin but to such common antigens as molds, epidermals, inhalants, and certain foods, but none to pollens. The relationship between delayed type of hypersensitivity in the hypogammaglobulinemic patient and clinical allergy is discussed. The possible significance of delayed type of reactions in routine skin testing is emphasized.

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FIELD EVALUATION OF RAGWEED POLLEN SAMPLERS

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INTRODUCTION

In 1953 and 1954, the Durham sampler was used in a survey of air-borne pollen grains and fungus spores made at thirty-five locations in New York State.[1] At several of these locations, identical samplers of other agencies were operated nearby, but at different heights, for sampling ragweed pollen. Comparisons of data revealed unexplained differences in ragweed pollen count. In general, the higher counts were obtained at the higher elevations. Since previous studies[2-8] had revealed high counts of pollen and spores at altitudes of several thousand feet, and as routine sampling is conducted at varying elevations, it seemed desirable to study the variation in ragweed pollen concentrations in the lowest few hundred feet of the atmosphere. To accomplish this, a cooperative project was undertaken by the State Botanist's Office of the New York State Museum and Science Service and the Meteorology Group of Brookhaven National Laboratory. Field studies were begun during the 1955 pollen season at the Laboratory, which is located in central Long Island, New York.

Sampling was conducted at eight levels, from 9 to 410 feet, on a 420-foot meterology tower, a triangular structure of open steelwork and constant dimensions from bottom to top. During analysis of the data, it was concluded that the sampling methods being used gave results that were influenced by a number of variables in addition to the concentration of ragweed pollen. Because of this, no conclusion was possible concerning the variation of concentration with height, but a number of experiments were initiated which were designed to give a better evaluation of the sampling methods.

EFFECTS OF WIND DIRECTION ON SLIDES

The 1955 sampling array included two Durham samplers mounted 2 feet apart on the railing at each of the eight tower levels. The mounting technique conformed with recommendations of the Pollen Survey Committee of the Research Council of the American Academy of Allergy.[9] One of each pair was

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mounted with the long axis of its slide in a north-south position, and the other sampler was mounted with the long axis in an east-west position. The greased slides were changed daily at 0800 from August 22 to September 9. Fig. 1 summarizes the data from the samples at the various levels.

Inspection of the pollen counts showed that at all levels the north-south (N-S) slide usually took more pollen than the east-west (E-W) slide. Throughout the sampling period, slide N-S took 9,226 grains, or 58 per cent of the total, while slide E-W took 6,628 grains, or 42 per cent of the total. Since wind direction was judged to be the only meteorological variable capable of producing

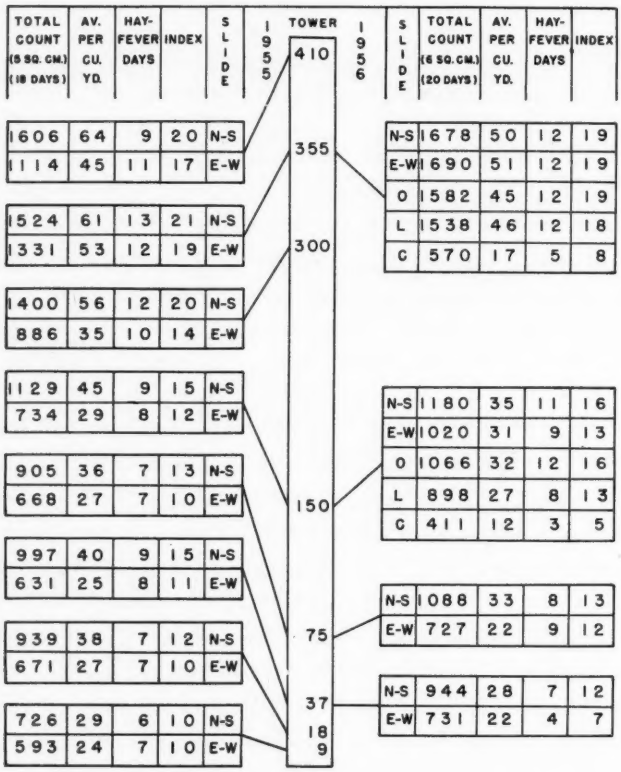

Fig. 1.—Comparison of data computed from Durham sampler pollen counts at different altitudes during 1955 and 1956. The figures in the total count column are not converted to a volumetric basis. Average per cubic yard is determined by multiplying the average number of pollen grains per square centimeter by 3.6. A hay fever day has an average of 25 or more grains per cubic yard. The ragweed pollen index is obtained by converting the data to a volumetric basis, then assigning one point for each hay fever day, one point for each 100 grains of pollen on the day of the highest count, and one point for each 200 grains of pollen in the seasonal total. "Slide" refers to the orientation of the slide. *N-S*, Slide with long axis pointing north and south; E-W, slide with long axis east and west; O, round slide in a circular holder; *L*, slide with long axis parallel to direction of air flow; *C*, slide with long axis at right angles to air flow. The tower heights are in feet.

these results, an analysis of the hourly wind data taken during the period was made. The 355-foot direction was chosen as representative. For each day, the percentage of hours during which a wind came from each of the four quadrants was computed. The percentages from north and south were then added, as were

the percentages from east and west. For the period as a whole, north or south winds prevailed 53 per cent of the time and east or west 47 per cent, which indicated that the difference in pollen capture might be related to the wind direction.

In 1956 the same slide orientation was repeated at each of four levels. Midway through the experiment, the positions (but not the orientation) of the samplers at each level were reversed with no change in the trend. Once again, at all except the top level, the N-S slides took considerably more pollen than the E-W slides beside them (Fig. 1), while the N-S wind direction again predominated. These data with respect to slide orientation are not in agreement with those reported by other investigators[10] who found little correlation between placement and pollen capture.

In order that the effect of slide orientation with respect to wind direction might be investigated better, another experiment was conducted in 1956. Pairs of Durham samplers were mounted on two wind vanes. One slide of each pair was placed with its long axis parallel to the vane; the other was placed with its long axis at right angles to the vane. Thus, slide L continuously had the air flow over its length while slide C had the flow over its width. The vanes were mounted at 150 and 355 feet, and the slides were changed daily for a twenty-day period. On seventeen of the twenty days, higher counts were obtained at both levels on the lengthwise (L) slides. As shown in Fig. 1, the total L slide count was more than double that of the C slide at the 150-foot level (a ratio of 2.2 to 1) and nearly three times as large (a ratio of 2.7 to 1) at 355 feet.

In 1957 a further check of this effect was made. Two wind tunnels were constructed and mounted approximately 4 feet above the ground in an open field near the tower. Each tunnel was 30 inches in diameter and 9 feet in length. Each had a two-speed fan mounted at one end to provide suction, and a totalizing anemometer was mounted inside to record the total air passage. In each tunnel, a lengthwise and a crosswise slide were mounted and changed daily for a thirteen-day period. The fan in tunnel 1 was run at its fast speed, giving an average air flow of 8.0 m.p.h., while the slow speed of the fan in tunnel 2 gave an average of 4.2 m.p.h. In tunnel 1, 1,568 grains were taken on slide L and 307 on slide C, a ratio of 5.1 to 1. In tunnel 2, 1,051 grains were collected on slide L and 395 on slide C, a ratio of 2.8 to 1.

Both the vane and the tunnel tests show that a slide oriented parallel with the air flow collects a great deal more pollen than one oriented at right angles and that the difference is greater at higher wind speeds. Since the reasons for this effect were believed to be purely aerodynamic, the flow patterns over the slides were investigated in a wind tunnel recently constructed at Brookhaven. Wind speeds in the tunnel may be varied from zero to 45 m.p.h. Oil-fog smoke is produced by a small generator and introduced into the tunnel to allow visual observation and photography of the air flow past objects under test. The sampling devices used in our field studies were tested at air speeds of 6 and 15 m.p.h., which are representative of the range of speeds encountered in actual field sampling.

Fig. 2 shows typical flow patterns over a microscope slide in the standard Durham holder. It can be seen that as the air approaches the blunt leading edge of the slide and its holder it is forced to diverge from its course. As shown in Fig. 2, *A* and *B*, a small rise occurs over the leading surface of the slide at 6 m.p.h., but a much larger one takes place at 15 m.p.h. Thus, as the speed of the air approaching the slide increases, the proportion of that air which is effectively sampled decreases and the pollen collected tends to deposit toward the rear of the slide. In spite of the decreased proportions of air sampled at increasing wind speeds, our field tests show that the greater volume of air sampled results in a greater over-all catch. Fig. 2, *C* and *D*, shows the flow patterns as the air passes over the short axis of the slide. At 6 m.p.h. the rise

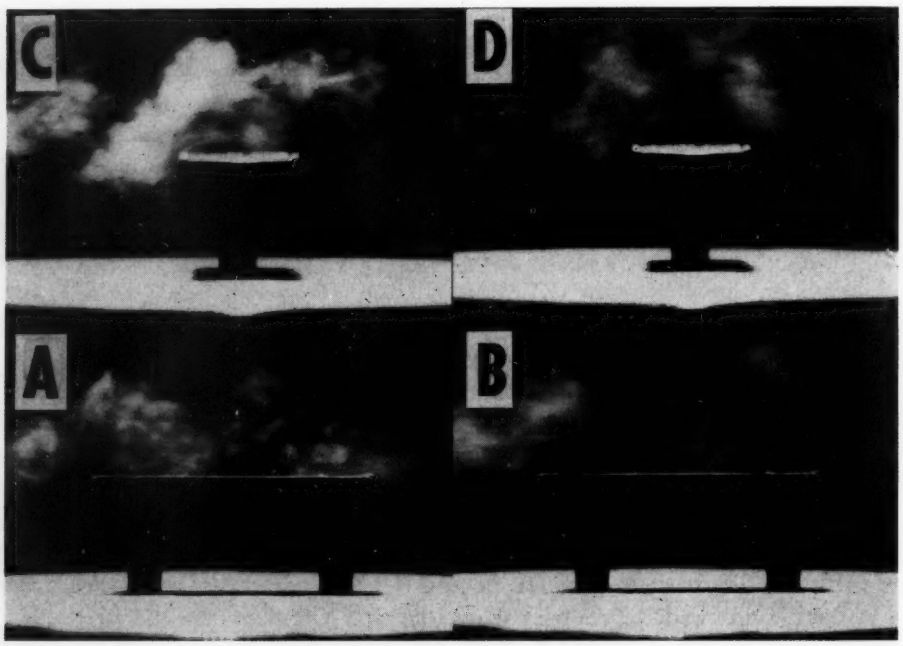

Fig. 2.—Wind-tunnel photographs of flow patterns over a slide in a Durham holder. Air flow is from left to right. *A*, Slide lengthwise, air speed 6 m.p.h. *B*, Slide lengthwise, air speed 15 m.p.h. *C*, Slide crosswise, airspeed 6 m.p.h. *D*, Slide crosswise, air speed 15 m.p.h.

over the leading surface of the slide carries the air past the middle before it descends, while at 15 m.p.h. the air is effectively lifted over the entire surface. These flow patterns explain the smaller catch obtained on a slide oriented across the wind.

These findings are further confirmed by counts which were made on the two halves of the lengthwise slides exposed in the tunnels in 1957. The front half of the slides took 1,026 grains, or 37 per cent of the total, while the back half captured 1,778, or 63 per cent of the total. These figures reinforce the observations that the air approaching the leading edge of the slide is forced to rise, carrying much of the pollen over the first inch or so of the slide. Thus, in an air flow over the long axis of the slide, most of the pollen is caught on the back half, while in an air flow over the short axis, much of the pollen passes

completely over the slide. This is confirmed by the much smaller catch on the slides oriented across the wind in the tests made in 1957 and by the approximately equal catches on both halves.

EFFECTS OF WIND SPEED ON SLIDES

As shown in Fig. 1, the amount of pollen collected on the slides in both 1955 and 1956 showed a fairly uniform increase from the bottom to the top of the tower, with the catch at the top about double that at the bottom. This might have been interpreted as indicating an actual increase in concentration with height had not simultaneous filter samples shown the opposite trend. It was soon realized, however, that the catch on the slides was a function not only of the concentration of pollen in the air but also of the amount of air which passed over the slide. Therefore, the slides exposed to the higher wind speeds at the top of the tower sampled a greater volume of air and caught a larger quantity of pollen.

In order to test this effect under more controlled conditions, slides were exposed in both 1956 and 1957 in the wind tunnels previously described. In 1956, the average tunnel air flows were 5.1 and 8.3 m.p.h., while their total pollen counts were 1,144 and 1,465 grains. In 1957, the air flows were 4.2 and 8.0 m.p.h., and the pollen catches were 1,446 and 1,875 grains. Thus, in both years, a 3 to 4 m.p.h. increase in wind speed gave about a 30 per cent increase in the pollen catch. Actually, the effect was even more marked in 1957 if we consider only the lengthwise slides. Here the total catches were 1,051 and 1,568 grains, an increase of 49 per cent in the faster tunnel.

The slides used in the vane samplers in 1956 give additional evidence. The lengthwise slide at 150 feet, where the wind speed averaged 4.7 m.p.h., took 898 grains, while that at 355 feet, exposed to an average speed of 6.0 m.p.h., took 1,538, a 71 per cent increase. The crosswise slides showed less difference–411 and 570 counts or a 39 per cent increase.

EFFECTS OF WIND SPEED ON FILTER SAMPLERS

In both 1955 and 1956, the slide samples on the tower were augmented by samples taken at several levels on Millipore (molecular membrane) filters mounted in filter holders of a type then in use at Brookhaven.[11] Air was drawn through the filters by an electric vacuum pump at the rate of 0.75 cubic feet per minute. In contrast to the slide samples, the filter counts showed a marked decrease with height in 1955 and a similar but less marked trend in 1956. This indicated that the increase of wind speed with height might have an effect on the filter samplers opposite to its effect on the slides.

In 1956, filter samplers were mounted in both wind tunnels and changed daily to check the effect of wind speed on their efficiency. The twenty-four hour period between changes proved to be too long, for the filters became so heavily loaded with dust, as well as pollen, that accurate counting was impossible and the data were discarded. In 1957 the experiment was repeated, with the filters changed automatically every six hours by the automatic programming filter

sampler.[12] On all but one of the eleven days, the filter in the slow tunnel caught considerably more pollen than the one in the fast tunnel. The totals for the period were 3,082 and 1,051 grains, a ratio of nearly 3 to 1, although the same volume of air was pulled through both filters and the concentrations of pollen in the volumes sampled must be presumed identical.

The reason for this effect may be readily explained. In order to collect a particle on a filter, the air containing that particle must be drawn through the filter by some suitable means of suction. If the filter holder opening is at right angles to the direction of air flow, the air must make a 90 degree turn to enter the filter. If the particles in the air are very small (less than 1 micron, for instance), they tend to follow the streamlines of the air faithfully at any normal speed. Larger particles also tend to follow the air flow into the filter if they are moving slowly. However, these larger particles, such as ragweed pollen, have enough momentum in an air flow of considerable speed that they tend to continue in their original direction when the air containing them turns to enter the filter. This effect is the same whether the filter holder is mounted upright, upside down, as has been previously suggested,[13] or on its side. The only difference, except during precipitation, is the negligible effect of gravity on the particle.

If the filter holder is mounted so that its opening is continuously oriented into the wind, good results will be obtained when the air flow through the filter is equal to the wind speed in the free air approaching the filter. If this is not the case, however, the system is subject to the serious errors of anisokinetic sampling discussed by Watson.[14] An excellent summary of the principles of isokinetic sampling is given by Wilcox.[15]

The molecular membrane filter has been used by several investigators for the collection of pollen.[13, 16-18] In contrast to other types of filter media, it has the advantage of retaining the collected material on its surface where it may be viewed under a microscope. Our experience, as well as that of others,[13] has shown that the recognition of collected ragweed pollen is feasible, provided the filters are not too heavily loaded with other particles. This consideration may limit the flow rate and exposure time for a given filter.

Tests have shown that the efficiency of this filter in retaining particles in the pollen size range is essentially 100 per cent.[13-17] Several experimenters have been misled by this into assuming that samples taken with the filter are therefore 100 per cent accurate. Due to the source of error just discussed, this is far from the case. In our experiments, for instance, the filter holders were mounted in an upright position normal to the horizontal air flow. The pull of 0.75 cubic foot per minute gave a linear flow of about 2 m.p.h. in contrast to the wind speeds which sometimes reached 20 m.p.h. Thus, it is evident that our filter system (and probably that of previous workers) is efficient only at very low wind speeds. Since this filter material is not adapted for high flow rates and since materials that are so adapted usually collect particles in the interstices of the material where they are not visible, it seems that the collection

of pollen on filters may not be the ideal way to obtain an accurate sample that some have believed it to be. This method is, in fact, subject to objections as serious as those to the gravity slide method.

DISCUSSION

Several previous investigators have realized that the current slide method of pollen sampling is grossly inaccurate.[5, 18-26] Our experiments show that the combined effects of wind speed and wind direction may introduce differences of over 500 per cent between adjacent samplers and that the number of hay fever days and the ragweed pollen index computed from such data may show wide variations, even though the same concentration is being sampled. Current practice in exposing slides without regard to these variables yields results which cannot be regarded as comparable from one station to the next or from

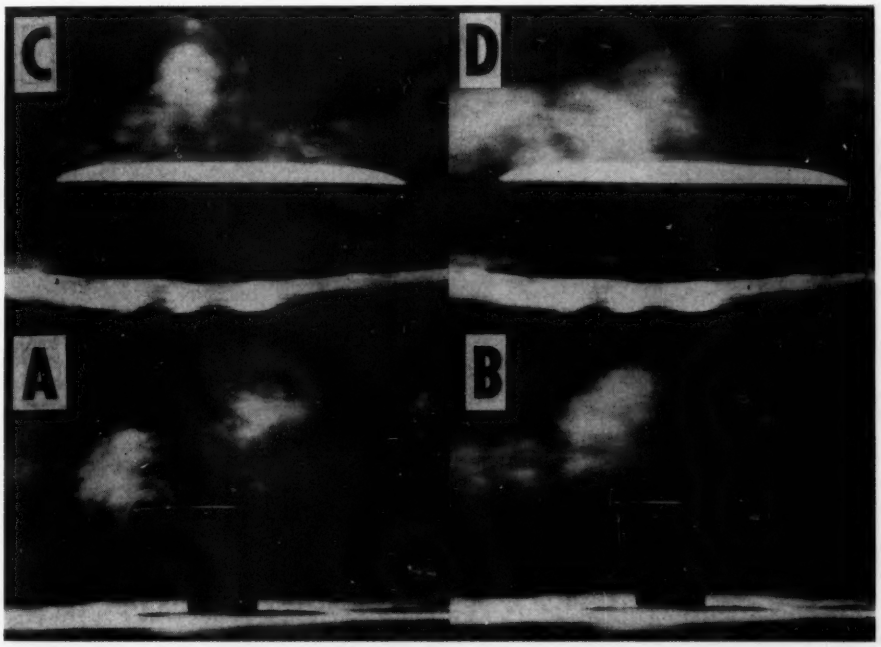

Fig. 3.—Wind-tunnel photographs of flow patterns over round slide holders. Air flow is from left to right. *A*, Model 1, air speed 6 m.p.h. *B*, Model 1, air speed 15 m.p.h. *C*, Model 2, air speed 6 m.p.h. *D*, Model 2, air speed 15 m.p.h.

one day to the next at the same station. Since comparability is essential in many applications of the pollen count, greater uniformity in sampling is desirable. If the Durham sampler continues to be used, a method should be established to ensure uniform orientation with respect to wind direction. This can easily be accomplished by mounting the sampler on an inexpensive wind vane. Uniform orientation with respect to air flow has been suggested previously[27-31] and is strongly recommended by us.

An alternate method of overcoming the directional bias is the use of a round or square slide in a suitable holder. A round slide holder was used in the 1956 field tests. Experiments have been conducted with other such models and will be continued, but as yet not enough data have been obtained to permit a proper evaluation of their effectiveness. Round slides are more difficult to mount and handle than the rectangular slides, but square slides give little difficulty.

Fig. 3, *A* and *B* shows the flow patterns over the original round-slide holder (Model 1) at 6 and 15 m.p.h. It is obvious that the shape is aerodynamically poor for efficient pollen collection. Fig. 3, *C* and *D* shows the flow patterns over a holder of improved design (Model 2) at the same wind speeds. In contrast to the previous model this holder allows the air to flow smoothly over the surface at both speeds. This holder, with square slides, will be tested in the field during the 1959 season.

Varying wind speeds as a source of error have also been recognized previously[24, 30, 32-34] and present a more difficult problem than direction. Attempts made by others to reduce or make uniform the rate of air flow over the slides have not resulted in satisfactory counts, since some of the pollen is apparently removed by the obstacles used. Perhaps the most suitable solution is to measure the wind speed or total air passage over the slide and to correct the count by a factor determined either theoretically or experimentally. We have developed an inexpensive anemometer for this purpose, and work is being done toward the determination of a usable correction factor.

Alternate Sampling Methods.—Sampling methods that do not involve slides and filters are still being studied, and these give promise of greater accuracy. Although final evaluations of these devices have not yet been made, brief descriptions may be helpful to other workers.

For short-period samples, the Rotorod sampler[25] is believed to be an efficient device. This sampler collects pollen by impaction on a pair of small rods whirled through the air at high speed by a small, inexpensive, battery-operated motor. Single units are not adaptable for long-period samples, since they would become badly overloaded, but a number of units may be operated in sequence by a timing device. A number of these samplers have been constructed at Brookhaven and are currently being tested.

Another method of sampling that shows some promise and is being tried by us is the collection of pollen on the side of a vertical cylinder, a method tested by Gregory.[36] The collecting side of the cylinder is kept oriented into the wind by a vane, while an anemometer is used to record the total air flow. Since the efficiency of this device falls off rapidly with decreasing wind speed and increasing cylinder size, only very small cylinders are useful at the lighter wind speeds.

Another device currently under design is an isokinetic sampler which will change its flow rate rapidly and automatically to conform to changes in the wind speed while the sampling intake is oriented into the wind by a vane tail. Although this sampler will be too complex and too expensive for general use, its efficiency should prove very high. It will be used as a standard for the evaluation of other sampling devices.

Numerous other samplers reported in the literature have not been tested by us. Some of these obviously suffer from the same faults as the devices tested. Others may be more efficient but, until a reliable basis for comparison is available, evaluation of the performance of any sampler is subject to many uncertainties.

SUMMARY

Our experiments have shown that pollen capture by the standard Durham sampler is determined by several factors, in addition to the concentration of pollen in the air. Aerodynamic factors are responsible for the greater collecting ability of the slide when its long axis is placed parallel to the air flow. Orientation of the slide with respect to wind direction may result in a 5 to 1 difference in catch between adjacent slides.

It was also found that the amount of pollen captured is a function of the amount of air passing over the slide as well as the concentration of pollen in the air. Thus, an increase of 3 to 4 m.p.h. in the wind speed may give more than a 50 per cent increase in the pollen captured. Since wind speed normally increases with height, elevation of the sampler above the ground determines the wind speed to which the slide is exposed and affects the count.

Tests of a filter sampler showed that this device was also affected by wind speed but in an opposite manner, since efficiency in capturing particles in the pollen size range decreases with increasing wind speed.

Improvements in the standard sampling technique might include vane orientation of the slide sampler and measurement of the air flow which could be used to correct the count, or the use of round or square slides with similar correction of wind speed. Work is continuing on the development and evaluation of other sampling devices.

Maynard E. Smith, Leader of the Meteorology Group at Brookhaven National Laboratory, and Irving A. Singer, Assistant Leader, assisted materially in the planning and conduct of these experiments. Donald M. Lewis of the State Botanist's Office counted all the samples and actively participated in the 1956 and 1957 programs. Louis H. Conger, Jr., formerly Acting Chief of the Bureau of Statistical Services of the New York State Education Department, assisted in the analysis of data. Herbert M. Mapes constructed several modified slide holders.

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IMMUNOLOGIC STUDIES OF RAGWEED-SENSITIVE PATIENTS

I. Specificity and Sensitivity of Hemagglutination Reactions

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THE development of techniques to demonstrate antibodies in vitro in sera of allergic patients has been the goal of many investigators for several decades. As early as 1925, Coca and Grove[1] were unable to demonstrate precipitins in the sera of allergic persons, although these sera did contain the skin-sensitizing antibody. Many other workers in this field have confirmed this discrepancy. It was believed by many that the lack of precipitin in the allergic sera was due either to the fact that we were dealing with a univalent or incomplete antibody in ragweed-sensitive patients or to the fact that our methods of determining precipitins were too insensitive to demonstrate small amounts of this type of antibody. Cannon and Marchall[2] and Cohen and Weller,[3] by using collodion particles coated with ragweed pollen extract, were able to show antibodies in vitro in some ragweed-allergic sera. This method was quite cumbersome and not readily reproducible. In more recent years, however, red blood cells instead of collodion particles have been successfully employed, and antigen can be either coated nonspecifically or linked chemically to these red blood cells. The addition of the specific antiserum produces a clumping of the red blood cells. This procedure was called indirect hemagglutination. It has been described as being 1,000 times more sensitive than the precipitin test.[4]

One method of hemagglutination was that first described by Boyden,[5] who coated antigens on red blood cells that had been previously treated with tannic acid. Several investigators[6-9] have used modifications of this method to measure antibodies in patients sensitive to pollens. For the most part, antibodies demonstrated by this method were found (in relatively low titers) primarily in the sera of patients who had received specific hyposensitization therapy. Feinberg and associates[9] noted positive hemagglutination reaction in 21 per cent of untreated patients and 67 per cent of patients treated with pollen extract.

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They also stated that there was elution of the antigen that was coated on the tannic-acid-treated red blood cells, which may have accounted for their relatively low titers.

Another method of hemagglutination, first described by Pressman and colleagues,[10] consisted of an actual chemical linkage between the red blood cell and the antigen by means of bis-diazotized benzidine. This technique was applied and first used for pollen antigens by Stavitsky and Arquilla.[11] It was more thoroughly studied by Gordon and associates,[12] who reported that the sera of all ragweed-sensitive patients, either treated or untreated, had antibodies demonstrable by this method and that this test was specific.

Recently, Beede, Rose, and Arbesman[13] used successfully the tanned-cell technique, as developed by Witebsky and Rose,[14] with animal sera and danders as antigens.

The purpose of our study was to determine the specificity and relationships of both the modified tanned-cell and bis-diazotized-benzidine (BDB) techniques. We used ragweed pollen as the antigen, sera from ragweed-sensitive patients, and antiragweed rabbit sera as the source of antibodies.

METHODS AND MATERIALS

Sources of Antibodies.—Samples of blood were collected under sterile conditions from 113 ragweed-sensitive patients from private practice and from the Allergy Clinic of the Buffalo General Hospital. The sera were kept frozen until ready for use. An additional group of sera was obtained from "non-ragweed-sensitive" allergic persons. Three groups of control sera were likewise gathered for our studies from (1) the serologic laboratory (hospitalized "sick" patients), (2) the blood bank (healthy persons), and (3) patients who had serum sickness and high hemagglutination titers against horse serum.

Antiragweed rabbit sera were prepared by several different means in order to obtain adequate precipitin and hemagglutinin titers. At first alum-precipitated ragweed extracts were injected subcutaneously in several sites; if, after a period of three to four weeks, trial bleedings failed to show adequate antibody response, other courses of immunization were carried out. These included intravenous injections of the alum-precipitated extracts and/or the saline ragweed extracts given three times weekly over a four-week period. One to two weeks following the last intravenous injection, a trial bleeding was again performed. If the titer was adequate, the animal was bled out. In many instances, however, three to four courses of intravenous immunization were necessary.

We also employed a method in which equal parts of a 15 per cent saline extract of ragweed pollen and Freund's complete adjuvant (0.1 ml. of each) were injected into the four footpads of the rabbit. Again, trial bleedings, titer determinations, and further immunization by both the intravenous and the intradermal routes were employed, when needed, to obtain the desired antibody response.

Despite all these intensive methods of immunization, several animals were unable to develop sufficient antibodies.

Sources of Antigens.—A 15 per cent extract of defatted short ragweed pollen in buffered saline* (without phenol) was prepared by first treating ragweed pollen with petroleum ether in a Soxhlet apparatus until the distillate was colorless. This "defatted" material was then extracted with a buffered saline solution in a shaking machine for forty-eight hours at room temperature. After Buchner and Seitz filtration, the extract was bottled in 30 ml. vials and placed in the freezer. It was used in all the experiments to be reported in this and subsequent presentations.

A 15 per cent June grass extract was prepared in a similar manner.

Horse serum was obtained from a local slaughterhouse and kept frozen until ready for use.

Materials for Tanned-Cell Hemagglutination Test.—Human group O, Rh negative, red blood cells were taken in standard ACD† solution.

Tannic acid (C. P. Baker—not fluffy) stock solution, 0.5 Gm. in 100 ml. of distilled water (1:200) was prepared fresh each week. Dilutions for immediate use were made with buffered saline, pH 7.2.

The pH 7.2 buffered saline was prepared by adding 20.4 Gm. NaCl, 4.45 Gm. Na_2HPO_4 (anhydrous), and 1.3 Gm. KH_2PO_4 to 3,000 ml. of distilled water. This solution was checked by a pH meter and adjusted if necessary.

The pH 6.4 buffer was prepared by mixing 26.7 ml. of M/15 Na_2HPO_4 to 73.3 ml. M/15 KH_2PO_4.

Materials for Bis-Diazotized-Benzidine Method.[12]—Normal rabbit red blood cells were collected from the ear vein into an equal volume of Alsever's solution‡ and stored at 4° C. for as long as ten days. The red blood cells, when needed, were separated by centrifugation and washed three times with normal saline.

Normal rabbit serum was collected from the same rabbit that supplied the erythrocytes.

Bis-diazotized benzidine (BDB) was prepared by dissolving 0.23 Gm. of benzidine in 100 ml. of water, to which was added 0.75 ml. of 12 N hydrochloric acid. Next, 0.175 Gm. of $NaNO_2$ was dissolved in about 5 ml. of water, and this was slowly added to the solution. The reaction was allowed to proceed for thirty minutes with intermittent stirring. Aliquots of the solution were then placed into 2 ml. vials, quick frozen at –78° C. in a dry ice–acetone bath, and stored at –20° C.

The 0.15 M pH 7.3 phosphate buffer was prepared by mixing 2.5 ml. of 0.15 M Na_2HPO_4 with 49 ml. of 0.15 M KH_2PO_4.

Method of Tanned-Cell Hemagglutination Test.—

1. *Washing red blood cells:* To 2.0 ml. of human group O, Rh negative, red blood cells was added 20 ml. of buffered saline, pH 7.2. The tube was

*Buffered saline solution:

Sodium chloride	0.5%
Sodium bicarbonate	0.825%.

†ACD = Acid citrate dextrose (Baxter).

‡Alsever's solution:

Glucose	2.05
Sodium citrate	0.8
Sodium chloride	0.42
Distilled water to 100 ml.	

inverted gently two to three times and then centrifuged for five minutes at 1,500 r.p.m. in an International refrigerated centrifuge, Model PR-2 with Head #284. The supernatant was removed, and the same process was repeated. The final washing was performed in a graduated tube with only 10 ml. of pH 7.2 buffer. The supernatant was again removed, and a 2.5 per cent suspension of red blood cells with buffered saline, pH 7.2, was prepared.

2. *Tanning red blood cells:* To one volume of 2.5 per cent red blood cell suspension was added an equal volume of tannic acid 1:20,000, diluted fresh for each experiment with pH 7.2 buffered saline from the 1:200 stock solution. This mixture was incubated for ten minutes in a 37 degree water bath and then centrifuged for five minutes at 1,000 r.p.m. The supernatant was removed and the packed red blood cells were resuspended with two volumes of buffered saline, pH 7.2, and centrifuged in the same manner as before. This process of washing the tanned cells was repeated once more, but with normal saline instead of buffered saline, and the supernatant was removed.

3. *Coating red blood cells with antigen:* The packed, washed, tanned red blood cells were resuspended by the addition of a mixture of equal volumes of pH 6.4 buffered saline and short ragweed extract (15 per cent defatted saline) diluted in 1 to 5 with buffered saline, pH 7.2. This red blood cell–antigen mixture was incubated for fifteen minutes in a 37° C. water bath and then centrifuged, as described previously, for five minutes. The supernatant was removed, and the red blood cells were resuspended with two volumes of 0.5 per cent normal rabbit serum (diluted with buffer saline pH 7.2) and centrifuged as before. This same process was repeated, and the cells were finally resuspended with 0.5 per cent normal rabbit serum to 1.25 per cent (approximately 4.5 ml.).

4. *Dilution of antisera:* Serial twofold dilutions (volume, 0.5 ml.) of antisera were made with 1 per cent normal rabbit serum. A separate pipette was used for each dilution.

5. *Test:* Tanned, coated red blood cells (1.25%) (volume, 0.1 ml.) were added to each tube of the serial dilutions of antisera (volume, 0.5 ml.) and to a control tube with normal serum. Another control consisted of tanned uncoated cells added to antisera.

The mixtures are shaken vigorously, the cells were allowed to sediment at room temperature for two hours, and the patterns were read. The tubes were again shaken and kept in the icebox overnight, and the patterns were read again.

6. *Reading of patterns:* The degree of hemagglutination was recorded from 4 plus to 0. A 4 plus reaction consisted of marked folding noted at the bottom of each tube. A reaction with less folding was considered a 3 plus reaction, and one with less tightly agglutinated cells was recorded as 2 plus. A 1 plus reaction presented a diffuse granular appearance, and a negative reaction had the appearance of a doughnut or ring (Fig. 1).

The difference in techniques between our method and those described by Feinberg and Flick and by Stavitsky are illustrated in Table I. We have

observed that the slight variations in technique from the Feinberg-Flick method gave us regularly reproducible results and no evidence of elution of antigen.

Bis-Diazotized-Benzidine (BDB) Method.—The same technique as that described by Gordon and co-workers[12] was employed in our studies, except that we used a different antigen. In preliminary studies it was determined that a 1:40 dilution (with pH 7.2 buffered saline) of a 15 per cent saline extract of defatted short ragweed pollen gave hemagglutination titers equivalent to their water-soluble ragweed extract 0.75 per cent. One other slight difference is that our test tubes were 12 mm. in diameter, whereas Gordon and associates used 10 mm. tubes.

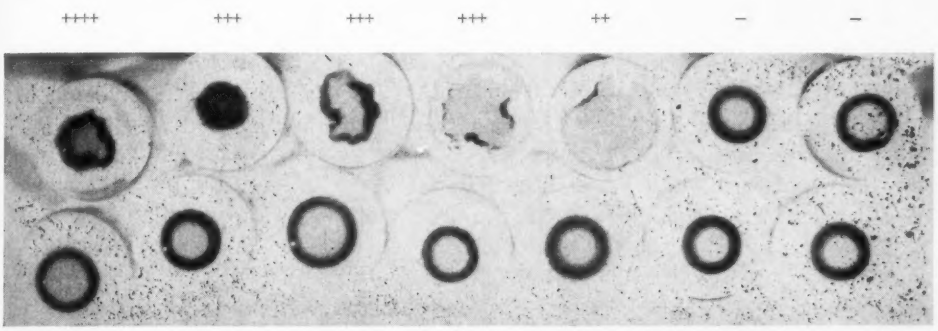

Fig. 1.—Tanned-cell hemagglutination patterns. (For method of reading patterns, see text.)

TABLE I. TANNED-CELL HEMAGGLUTINATION METHODS FOR RAGWEED ANTIGENS

	FEINBERG AND FLICK	STAVITSKY AND ARQUILLA	PRESENT METHOD
1. Red blood cells	Human group O, Rh negative, in Alsever's solution	Sheep	Human group O, Rh negative, in ACD
2. Washing red blood cells	Normal saline; then resuspended in 7.2 buffered saline	Normal saline	7.2 buffered saline
3. Coating red blood cells	Red blood cells first resuspended in pH 6.4 buffer, then antigen added	Red blood cells resuspended in buffered saline; then added to mixture of 1 volume of antigen and 4 volumes of 6.4 buffer	Red blood cells suspended in *mixture* of equal volumes of antigen and pH 6.4 buffer
4. Incubation	15 minutes at 37° C.	10 minutes at room temperature	15 minutes at 37° C.
Washing coated red blood cells	Normal rabbit serum 1:200 in normal saline twice	Normal rabbit serum 1:100 in normal saline once	Normal rabbit serum 1:200 in 7.2 buffered saline twice
Resuspension	Normal rabbit serum 1:200 in normal saline 1.8%	Normal rabbit serum 1:100 in normal saline 2.5%	Normal rabbit serum 1:200 in 7.2 buffered saline 1.25%
5. Volume of antisera used	0.1 c.c.	0.5 c.c.	0.5 c.c.
6. Volume of coated cells added	0.05 c.c.	0.05 c.c.	0.1 c.c.

The recording of the patterns with the BDB technique was different from the tanned-cell patterns. A negative test was recorded only when the cells formed a compact button on the bottom of the tube. A 1 plus reaction was evidenced by a small doughnut or ring. A 2 plus reaction had a very large ring. A 3 plus and a 4 plus reaction consisted of folding of the edges and strong agglutination of cells. These varying degrees of reaction are shown in Fig. 2.

Tanned-Cell Method for Horse Serum Antigen.—A modified technique, described in detail in another publication,[15] was employed with horse serum as the antigen to coat the red blood cells.

Inhibition Tests.—

1. Varying dilutions of short ragweed pollen extract in 1 per cent normal rabbit serum (0.5 ml.) were added to 0.1 ml. of antiragweed rabbit serum. This was set up in several rows, with a different dilution of the antiragweed rabbit serum in each row. The mixtures were shaken and remained at room temperature for two hours. Tanned, coated red blood cells (0.1 ml.) were added to each tube. The tubes were shaken, and the patterns were recorded as before.

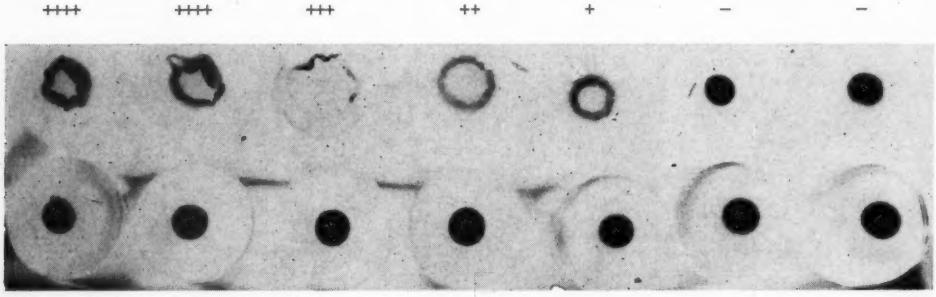

Fig. 2.—BDB hemagglutination pattern.

A similar technique of mixing varying dilutions of ragweed extract and antiragweed rabbit serum was used for the BDB method to demonstrate specific inhibition.

Various antigens, other than ragweed, were likewise used in these inhibition experiments.

2. Another type of inhibition test consisted of mixing equal parts of the pollen antigen and antirabbit serum. The mixture was incubated at room temperature for one hour, and the serial dilutions with 1 per cent normal rabbit serum were made of this mixture. Ragweed-coated tanned red blood cells were then added to these various tubes, and the results were recorded as previously described. The specificity of the test was determined by using June grass pollen extract as an inhibiting antigen and also for coating red blood cells.

3. Absorption tests were also carried out. Human serum from ragweed-sensitive patients (1.5 ml.), diluted 1 : 5 with 1 per cent normal rabbit serum, was added to 0.25 ml. of packed red blood cells treated with bis-diazotized benzidine

and linked to ragweed extract 1:40. This mixture was shaken and incubated at room temperature for one hour. Following centrifugation (as with the regular BDB technique), the supernatant was removed. An aliquot was saved, and the remainder of the supernatant was again exposed to red blood cells linked to ragweed extract by BDB. The same process as with the first absorption was repeated a total of five times. The aliquot following each absorption was then set up in duplicate serial dilutions with 1 per cent normal rabbit serum. BDB and tanned-cell hemagglutination tests were then performed by the technique described earlier for the unabsorbed serum and the various aliquot samples.

This same procedure was performed, absorbing the human ragweed serum with tanned red blood cells coated with ragweed extract.

Controls for this type of test consisted of absorbing the human serum with red blood cells treated with BDB (or tannic acid) and linked to horse serum rather than to ragweed antigen and absorbing with red blood cells untreated and uncoated.

RESULTS

Hemagglutination Tests.—

Antiragweed rabbit sera: The sera of twenty-four rabbits, immunized by various means as previously described, were tested for their hemagglutination activity. Table II shows the titers obtained in several of these animals by hemagglutination and by the usual precipitin tests. Seventeen of the twenty-four animals developed hemagglutination titers, ranging from a low of 2,000 to a high of 256,000. Although there was a close correlation (within two dilution steps) in titers obtained by the tanned-cell and BDB methods in most instances, several sera had high BDB and low tanned-cell titers and some had high tanned-cell and low BDB titers.

TABLE II. HEMAGGLUTINATION AND PRECIPITIN TITERS OF ANTIRAGWEED RABBIT SERA

RABBIT NO.	TANNED-CELL TITERS*	BDB TITERS*	PRECIPITIN END POINTS†
4	128,000	256,000	1-1,000
12	32,000	256,000	1-100
24	128,000	128,000	1-10,000
44	16,000	32,000	1-10,000
388	4,000	16,000	1-1,000
500	32,000	32,000	1-10
386	4,000	2,000	1-10
407	16,000	2,000	1-1,000
64	32,000	128,000	1-1,000
413	8,000	16,000	1-100

*Reciprocal of highest dilution of antiserum which showed positive hemagglutination reaction.

†Highest dilutions of antigen with constant amount of undiluted antiserum, which showed a positive precipitin reaction.

Human sera: The sera of 113 hay fever (ragweed-sensitive) patients were tested by both techniques of hemagglutination, as were sixty-nine sera from the serologic laboratory (hospitalized patients), ninety-five sera from the blood

bank (healthy persons), sera from forty-nine patients who had serum sickness and high titers by hemagglutination with horse serum, and sera from nineteen allergic patients supposedly nonallergic to ragweed pollen. Table III shows the results obtained with these various groups of sera. Of the 113 ragweed-sensitive patients, twenty-one had never received any type of previous hyposensitization therapy; of the remainder, none had received any previous treatment for at least two to four months. Almost 100 per cent of these sera showed positive BDB hemagglutination titers, and 87 per cent showed positive titers by the tanned-cell method. There was no apparent difference between the sera of the untreated and the previously treated patients. If, however, we considered a significant titer for the BDB test to be 1-80 or greater and for the tanned-cell test, 1-20 or greater, then the percentage of positives would be considerably lower. (Table III—figures in parentheses.)

TABLE III. HEMAGGLUTINATION TITERS

SOURCE OF SERA	BIS-DIAZOTIZED-BENZIDINE METHOD			TANNED-CELL METHOD		
	NUMBER OF PATIENTS	NUMBER POSITIVE	PER CENT POSITIVE	NUMBER OF PATIENTS	NUMBER POSITIVE	PER CENT POSITIVE
Blood bank	92	42 (18)*	46 (19)	95	13 (10)†	14 (11)
Serology laboratory	69	35 (12)	50 (17)	63	12 (12)	19 (19)
Serum sickness patients	38	30 (14)	80 (37)	49	11 (8)	22 (16)
"Allergic" controls	16	12 (5)	75 (31)	19	6 (4)	32 (21)
Ragweed-sensitive patients						
A‡	92	90 (71)	98 (77)	92	80 (63)	87 (68)
B§	21	21 (14)	100 (67)	21	18 (11)	85 (57)

*BDB titers of 80 or greater.

†Tanned-cell titers of 20 or greater.

‡Ragweed-sensitive patients who had received specific hyposensitization therapy in previous years.

§Ragweed-sensitive patients who had never received specific hyposensitization therapy.

In the allergic group of patients who supposedly were not allergic to ragweed, there was still a high percentage of positive titers (75 per cent and 32 per cent by the BDB and tanned-cell methods, respectively). However, all of these patients were retested with a stronger ragweed extract (10,000 PTA/n) intradermally, and it was found that three patients who demonstrated positive hemagglutination titers by the tanned-cell method also had positive skin tests, and two patients with positive BDB titers likewise had positive skin tests. Hence, if we were to eliminate these patients from the group, the positive hemagglutination titers would be 19 per cent and 71 per cent by the tanned-cell and BDB methods, respectively.

The incidence of positive reactions in the control groups (the group of sera obtained from hospitalized patients, from the serologic laboratory, from blood donors from the blood bank, and from patients who had serum sickness

and a high hemagglutination titer against horse serum) are likewise shown in Table III. It will be noted that there is a higher incidence of positive reactions in both groups by the BDB method than by the tanned-cell method. Hence, in summary, in all the various control groups of sera tested, the BDB test showed positive reactions in approximately 50 per cent, whereas the tanned-cell test revealed positive reactions in only about 18 per cent.

Comparative titers of pretreatment sera of ragweed-sensitive patients: It can be seen that in some instances the BDB titer may be relatively high and the tanned-cell titer low for the same serum (Fig. 3). Of these seventy-two male and forty-one female subjects tested, the BDB titer was higher in seventy-five (67 per cent), the tanned-cell titer was higher in eighteen (16 per cent), and the same titers were obtained in another eighteen (16 per cent).

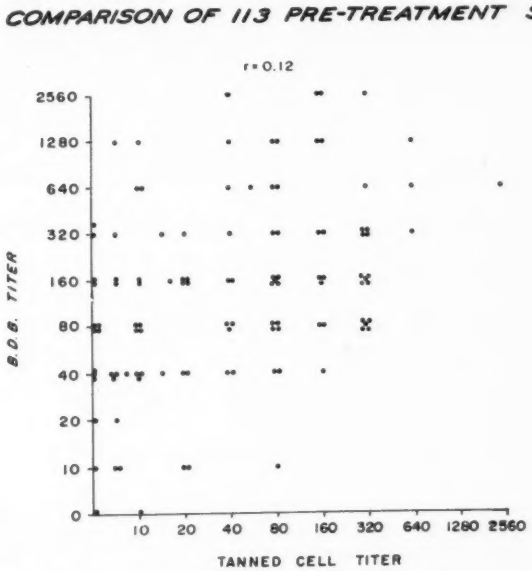

Fig. 3.—The actual hemagglutination titers as determined by the bis-diazotized-benzidine method and the tanned-cell technique in the same samples of sera from ragweed-sensitive patients prior to therapy. The numbers are the reciprocal of the highest dilution of the patient's sera that produce a positive hemagglutination reaction.

r = Correlation coefficient, which in this instance means that the value of 0.12 is not statistically significant.

Relationship of age distribution and hemagglutination titers: The number of patients in each age group and the average hemagglutination titers as determined by the BDB and tanned-cell tests are shown in Table IV. It appears that

TABLE IV. SUMMARY OF TITERS* ACCORDING TO AGE DISTRIBUTION

AGE	NUMBER OF PATIENTS	GEOMETRIC MEAN TITER	
		TANNED-CELL	BDB
0-10	6	56.6	320
11-20	23	39.4	165
21-30	31	37.1	125
31-40	24	23.1	109
41-50	14	32.7	160
51-60	10	18.2	211

*Reciprocal of highest dilution of serum which produced positive hemagglutination.

there is a gradual reduction in BDB titers with age up to the age of 40, and then there is an increase. When these findings were studied statistically, however, there was no real difference in the mean titers and the age of the patient.

Relationship of duration of symptoms and hemagglutination titers: The sera studied were obtained from patients who had had symptoms for one to fifty years. The duration of symptoms in years and the number of patients in each group, along with their respective titers, are listed in Table V. No apparent relationship was noted between the duration of symptoms and geometric mean titers measured by either the tanned-cell or the BDB method.

TABLE V. COMPARISON OF PRETREATMENT TITERS* TO DURATION OF SYMPTOMS (NUMBER OF YEARS OF PREVIOUS SYMPTOMS)

PREVIOUS SYMPTOMS (NUMBER OF YEARS)	NUMBER OF PATIENTS	GEOMETRIC MEAN TITER	
		TANNED-CELL	BDB
0-5	27	35.5	132.6
6-10	27	28.3	152.0
11-15	21	20.9	160.0
16-20	8	46.3	67.0
21-25	16	66.8	153.0
Over 25	12	19.2	151.0

*Reciprocal of highest dilution of serum which produced positive hemagglutination.

Relationship of number of years of previous specific hyposensitization therapy to the hemagglutination titers: Twenty-one of the 113 patients had never had previous therapy, whereas twenty-three had undergone treatment for six to ten years and eight patients had received treatment for more than ten years. As can be seen from Table VI, there is no apparent correlation between the number of years of previous treatment and either the BDB or the tanned-cell titers. It appears that the sera of those patients with no previous treatment had lower titers, but here again the finding is not statistically valid.

TABLE VI. COMPARISON OF NUMBER OF YEARS OF PREVIOUS TREATMENT TO TITERS*

NUMBER OF YEARS OF PREVIOUS TREATMENT	NUMBER OF PATIENTS	GEOMETRIC MEAN TITER	
		TANNED-CELL	BDB
0	21	12.8	96.6
1	18	34.7	205.9
2	11	73.6	173.6
3	19	66.3	158.2
4-5	11	42.7	150.1
6-10	23	27.3	124.3
Over 10 years	8	12.3	190.3

*Reciprocal of highest dilution of serum which produced positive hemagglutination.

Relationship of time interval between last injection of pollen extract and the hemagglutination titers: Twenty-one patients had received no previous injections, and fourteen had received their last injections more than two years prior to the pretreatment bleeding. Eight patients, however, had been given injections two to four months prior to the time their sera were obtained. As can be seen from Table VII, the titers of sera obtained from patients who had never received specific hyposensitization therapy are lower than those of sera from patients who had previously received treatment. There were significant

TABLE VII. RELATION OF TIME OF LAST INJECTION OF ANTIGEN TO PRETREATMENT TITER*

MONTHS SINCE LAST INJECTION	NUMBER OF PATIENTS	GEOMETRIC MEAN TITER	
		TANNED-CELL	BDB
Not previously injected	21	13.8	94.4
More than 24	14	22.5	113.1
9 to 24	13	26.0	110.1
8	12	36.9	124.7
7	10	44.1	139.3
6	18	33.6	345.6
5	15	70.2	192.5
2 to 4	8	46.3	103.7

*Reciprocal of highest dilution of serum which produced positive hemagglutination.

differences between the titers of sera from those patients who received their last injection five and six months (tanned-cell and BDB, respectively) previous to taking of the samples and those from patients who had received no treatment prior to the time their sera were secured.

Specificity of Hemagglutination Tests.—

1. Antiragweed rabbit sera were tested by these two techniques. The cells were coated with another pollen antigen, June grass. It is interesting to note (Table VIII) that there was some evidence of hemagglutination when June

TABLE VIII. TANNED-CELL HEMAGGLUTINATION TEST (CROSS REACTIONS BETWEEN RAGWEED AND JUNE GRASS)

	RABBIT ANTISERA			
	ANTIRAGWEED	ANTI-JUNE GRASS	ANTITIMOTHY	ANTIPLANTAIN
	Red Blood Cells Coated With Ragweed Extract			
Titer	5,000	80	Not done	Not done
	Red Blood Cells Coated With June Grass Extract			
Titer	640	10,000	5,000	640

See text for method.

TABLE IX. INHIBITION OF HEMAGGLUTINATION REACTION OF ANTIRAGWEED RABBIT SERUM (NO. 4) AND RED BLOOD CELLS COATED WITH RAGWEED AND JUNE GRASS EXTRACTS BY THE CORRESPONDING AND HETEROLOGOUS ANTIGENS

SUBSEQUENT DILUTION OF SERUM-ANTIGEN MIXTURES IN 1 PER CENT NORMAL RABBIT SERUM	ANTIRAGWEED RABBIT NO. 4 SERUM DILUTION 1:50					
	RED BLOOD CELLS COATED WITH RAGWEED			RED BLOOD CELLS COATED WITH JUNE GRASS		
	JUNE GRASS EXTRACT	RAGWEED EXTRACT	NORMAL RABBIT SERUM (CONTROL)	JUNE GRASS EXTRACT	RAGWEED EXTRACT	NORMAL RABBIT SERUM (CONTROL)
1:100	++++	--	+++	--	--	+++
1:200	++++	--	+++	--	--	+++
1:400	++++	--	+	--	--	+
1:800	++	--	+	--	--	+
1:1,600	+	--	+	--	--	+
1:3,200	+	--	+	--	--	+
1:6,400	+	--	+	--	--	+
1:12,800	+	--	+	--	--	--
1:25,600	+	--	+	--	--	--
1:51,200	--	--	--	--	--	--
1:102,400	--	--	--	--	--	--
1:100 (control)	--	--	--	--	--	--

See text for method.

grass was used as the antigen. Inhibition tests using anti-June grass and anti-ragweed rabbit sera and the corresponding homologous and heterologous antigens were also performed (Table IX). It can be observed that the ragweed antigen completely inhibits the reaction between the antiragweed serum and the red blood cells coated with both June grass and ragweed antigen, whereas the June grass antigen inhibits only the reaction with the June grass-coated cells but not with the ragweed-coated cells.

2. Red blood cells were coated with June grass and tested with the human ragweed sera. Only one patient, who had positive skin tests and was clinically sensitive to grasses, showed positive hemagglutination titers (Table X).

TABLE X. CROSS REACTIONS BETWEEN RAGWEED AND JUNE GRASS IN HEMAGGLUTINATION (TANNED-CELL) REACTIONS IN RAGWEED-SENSITIVE SERA

PATIENT	TANNED-CELL TITER	
	RAGWEED	JUNE GRASS
B. G.	160	0
M. B.	320	0
M. F.	40	40*
B. S.	40	0
J. D.	5	0
W. K.	160	0
G. B.	80	0

*Also had grass symptoms and positive skin test.

TABLE XI. SPECIFIC INHIBITION BY HOMOLOGOUS ANTIGEN OF HEMAGGLUTINATION TEST (TANNED-CELL)

DILUTIONS OF INHIBITING RAGWEED EXTRACT (0.5 ML.)	DILUTIONS OF ANTIRAGWEED RABBIT SERUM (0.1 ML.)		
	1:100	1:200	1:400
1:1,600	--	--	--
1:3,200	+	--	--
1:6,400	+	--	--
1:12,800	+	+	--
1:25,600	+	+	+
0	+	+	+
(1:10 normal rabbit serum)	--	--	--

TABLE XII. SPECIFIC INHIBITION BY HOMOLOGOUS ANTIGEN (RAGWEED POLLEN EXTRACT), BDB METHOD

DILUTIONS OF INHIBITING ANTIGEN RAGWEED POLLEN EXTRACT IN NORMAL RABBIT SERUM 1:100	DILUTIONS OF ANTIRAGWEED RABBIT SERUM					
	1:10	1:20	1:40	1:80	1:160	1:320
1:100	--	--	--	--	--	--
1:200	--	--	--	--	--	--
1:400	+	--	--	--	--	--
1:800	+	--	--	--	--	--
1:1,600	+	+	--	--	--	--
1:3,200	+	+	+	--	--	--
1:6,400	+	+	+	+	--	--
1:12,800	+	+	+	+	+	--
1:25,600	+	+	+	+	+	--
1:51,200	+	+	+	+	+	+
1:204,800	+	+	+	+	+	+
0	+	+	+	+	+	+
1:10	--	--	--	--	--	--

Inhibition Tests.—Specific inhibition tests, as described in our discussion of methods and materials, were employed with both the BDB and the tanned-cell tests. The results of inhibition tests are shown in Tables XI and XII. It can be seen that there is a specific inhibition with the homologous antigen and that it falls in a linear pattern. As little as 1:1,600 dilution of the 15 per cent ragweed pollen antigen is required to prevent the hemagglutination of a 1:100 dilution of antiragweed rabbit serum.

Absorption Tests.—Inasmuch as there was very little correlation between the titers of the human sera determined by the BDB and tanned-cell methods, we considered the possibility that two different types of antibody were being measured, due perhaps to the fact that different fractions of antigen (pollen extract) attach to a tanned cell or linked by bis-diazotized benzidine to the red blood cells. Sera from four different patients were studied. Two of these sera had high BDB titers and relatively low tanned-cell titers. The other two sera had higher tanned-cell titers and lower BDB titers. An example of an absorption test is shown in Table XIII.

As can be seen, the coated tanned cells absorb out all the antibodies demonstrable in tanned cell tests and benzidine in one or at most two absorptions, but the BDB antibody requires three or sometimes four absorptions before complete neutralization is noted. The converse experiment was performed; that is, the same human antiserum was absorbed by red blood cells linked to ragweed extract by bis-diazotized benzidine. Aliquots of each absorption were then tested by both the BDB and the tanned-cell methods. In these experiments the BDB-coated cells were more effective in removing the antibody and neutralized both the tanned cells and the bis-diazotized benzidine in one or two absorptions. The results suggest that the antibodies playing the primary role in the two types of tests may not be the same.

DISCUSSION

Almost all the sera from ragweed-sensitive patients showed positive hemagglutination titers by either the BDB or the tanned-cell method. This would indicate that some type of antibody against ragweed is measured by these techniques. The inhibition tests have shown the reaction to be specific. Other pollens cannot produce the same effect in persons sensitive only to ragweed.

Unfortunately, we found no correlation between the BDB and tanned-cell titers in the same sera. This difference in results could be due to the fact that other antigen-antibody reactions play the decisive roles in the two types of tests. The absorption tests did not clearly establish this point, since absorption of the patient's sera with either BDB- or tanned-cell–treated cells eventually removed all the antibody detectable by either method. However, all the antigens may have been coated on the red blood cells, but it may not necessarily have taken a complete part in the antigen-antibody reaction as evidenced by visible hemagglutination.

Our studies revealed a much higher incidence of positive results in various control groups tested by bis-diazotized benzidine than reported by Gordon and

TABLE XIII. INHIBITION (ABSORPTION) EXPERIMENT. SERUM FROM RAGWEED-SENSITIVE PATIENT ABSORBED WITH RED BLOOD CELLS TANNED AND COATED WITH RAGWEED POLLEN EXTRACT. ALIQUOTS FOLLOWING EACH ABSORPTION TESTED BY DIRECT HEMAGGLUTINATION BY BOTH BDB AND TANNED-CELL METHODS

DILUTIONS OF SERUM IN NORMAL RABBIT SERUM 1:100	UNABSORBED		FIRST ABSORPTION		SECOND ABSORPTION		THIRD ABSORPTION		FOURTH ABSORPTION	
	TANNED-CELL	BDB	TANNED-CELL	BDB	TANNED-CELL	BDB	TANNED-CELL	BDB	TANNED-CELL	BDB
1:5	+++	++++	--	+	--	+	--	+	--	--
1:10	+++	+++	--	+	--	+	--	+	--	--
1:20	+++	+++	--	+	--	+	--	--	--	--
1:40	++	++	--	+	--	+	--	--	--	--
1:80	++	++	--	+	--	--	--	--	--	--
1:160	++	++	--	+	--	--	--	--	--	--
1:320	++	+	--	--	--	--	--	--	--	--
1:640	+	+	--	--	--	--	--	--	--	--
1:1,280	+	+	--	--	--	--	--	--	--	--
1:2,560	--	+	--	--	--	--	--	--	--	--
Normal rabbit serum 1:5	--	--	--	--	--	--	--	--	--	--

associates,[12] who obtained negative reactions in all of the twenty-two "normal" sera they tested. We followed exactly the methods described by these workers. Through the kind cooperation of Dr. Bram Rose, two of us went to the Montreal Laboratory for several days to observe, first hand, the details of this test. We were able to reproduce their results. We exchanged blind samples of sera in each of the two laboratories, and the results obtained were quite comparable. The one difference in our methods is the antigen involved. We found that the 1:40 dilution of our 15 per cent defatted saline extract gave results equivalent to the water soluble extract (WSR) which Gordon and colleagues used.

We are convinced, therefore, that our technique is correct and that our findings are valid and would indicate that in our hands BDB method has a lower specificity than does the tanned-cell technique. Patients with a positive tanned-cell titer are more likely to be ragweed sensitive than those with a positive BDB titer. Only 14 per cent of our random control group showed positive tanned-cell titers.* It was noted that there was no significant difference in the percentage of positive reactions between the sera obtained from the blood bank (supposedly healthy persons) and the sera obtained from the serologic laboratory (hospitalized sick patients). This would indicate that disease processes had no effect on the hemagglutination titer produced by ragweed-coated or -linked cells. However, there was increased evidence of positive reactions, especially apparent by the BDB method, in those patients with such hypersensitive states as serum sickness or atopic disease.

Moreover, we found that although higher titers were usually obtained by the BDB method, the tanned-cell method seemed to be more reproducible and less likely to give the inconsistent results that we sometimes found by the BDB method. There was no evidence of elution of antigen from the tanned red blood cells, as was reported by Feinberg and Flick. This could be explained by either the different antigen used for coating the cells or the slight but significant changes in our methods of performing this test.

There is no correlation between the age of the ragweed-sensitive patient and the hemagglutination titer of his serum, the length of time the patient had symptoms, or the amount of previous treatment. However, the titers were definitely higher in those patients who had received injections five to six months previously, as compared to those patients who had never had specific treatment. This type of antibody which we measured by these methods is present in almost all ragweed-sensitive patients, regardless of age, duration of symptoms, or number of years of treatment. Furthermore, as will be reported in our next article, there was a definite increase in these antibody titers following intensive pollen therapy.

SUMMARY AND CONCLUSIONS

1. The sera from patients with ragweed sensitivity, both treated and untreated, contain substances which, when added to red blood cells coated or linked

*Although the patients from whom random serum samples were obtained were not interviewed or skin-tested for ragweed sensitivity, it is difficult to conceive that 46 per cent would be sensitive to ragweed as determined by the BDB technique. A more realistic figure of 14 per cent positive, as shown by the tanned-cell method, could conceivably have been from ragweed-sensitive patients.

to ragweed extract, cause a clumping of these cells (indirect hemagglutination).

2. Two methods, namely, the tanned-cell and bis-diazotized-benzidine methods, are suitable for demonstrating the particular antigen-antibody reaction. The results obtained by the two methods do not appear to correlate.

3. The BDB method usually gives higher titers than does the tanned-cell method in the sera of ragweed-sensitive patients. However, it also gives positive reactions to a much greater percentage of "random controls."*

4. The tanned-cell method, in our experience, is reliable, reproducible, and simpler to execute than is the BDB technique.

We wish to thank Mrs. Hazel Popp and June Spohr for their technical assistance, Dr. Edward Dowd of the Roswell Park Memorial Institute for his statistical evaluation of our results, and Mrs. Walter P. Stafford, Jr., for financial aid.

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*After submitting this article for publication, we received thirty-five sera from patients living in Israel who had never been exposed to ragweed pollen. Only one of these sera showed a positive hemagglutination titer by the tanned-cell technique in a dilution of 1:10. However, twenty-four of the thirty-five sera produced positive titers by the BDB method. Five of these thirty-five sera had titers greater than 1 to 40.

IMMUNOLOGIC STUDIES OF RAGWEED-SENSITIVE PATIENTS

II. Effect of Specific Hyposensitization Therapy on Hemagglutinating Antibody Titers

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IN A previous article[1] we studied the specificity and sensitivity of the hemagglutination test as carried out by the tanned-cell and the bis-diazotized-benzidine techniques. The effect of specific hyposensitization with ragweed pollen extracts on the hemagglutinating titers of sera from patients suffering from ragweed hay fever after treatment but before the onset of the ragweed pollen season and after the ragweed pollen season will be presented here. Sera were taken prior to the specific therapy (pretreatment). The effect of the inhalation of ragweed pollen during the hay fever season, the amount of treatment (amount of pollen extract injected), and/or the number of injections on these hemagglutination titers will also be reported.

Feinberg and colleagues[2] demonstrated that there was a higher hemagglutination titer following hyposensitization therapy (by the tanned-cell technique). Gordon and associates,[3] using the bis-diazotized-benzidine (BDB) technique, likewise showed an increase in titer in four patients from whom they had both pretreatment and posttreatment blood samples. They and other investigators[4-6] showed that serum from treated patients had higher titers than those from patients who were not given specific hyposensitization therapy. We extended this work by comparing both techniques in a larger number of patients and observing the titer changes in the same patient.

METHODS AND MATERIALS

Sera were collected, with sterile precautions, from 113 patients with ragweed hay fever who had not had any specific hyposensitization therapy for at least four months prior to the initial bleeding. These sera were stored in the freezer until ready for testing. Of these 113 patients, only sixty-nine were available for bleeding prior to the onset of the ragweed pollen season (posttreatment I). Forty-five of these patients also were bled and sera were collected

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after the ragweed pollen season. Preseason (posttreatment I) and postseason (posttreatment II) sera were obtained from an additional eight ragweed-sensitive patients who received no specific hyposensitization therapy. These "controls" were used to determine whether the inhalation of pollens had any effect on their hemagglutinating titers.

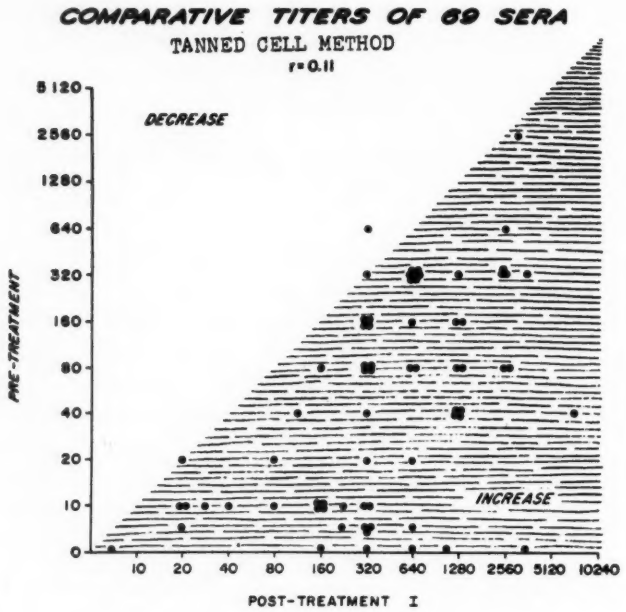

Fig. 1.

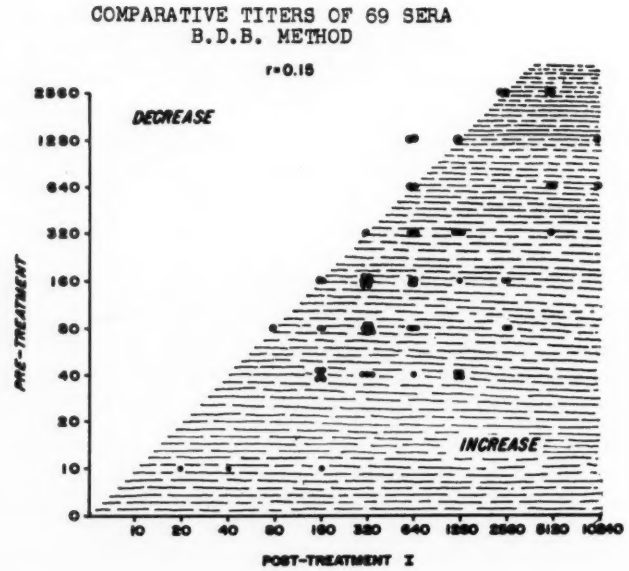

Fig. 2.

Figs. 1 and 2.—Numerals indicate the reciprocal of the highest dilution of serum which produces a positive hemagglutination reaction. All points in the shaded areas indicate an increase in titer following specific hyposensitization therapy.

The tanned-cell and BDB hemagglutination techniques were employed in these studies as described in our previous article.[1]

Hemagglutination titers of the various samples of sera from a particular patient were measured at the same time. These tests were done at least in duplicate by both methods.

RESULTS

Comparison of Pretreatment Sera and Posttreatment I (Preseason) Sera.— Figs. 1 and 2 compare the titers of sixty-nine sera taken prior to injection treatment with ragweed pollen extract and after the patients had received injection treatment but prior to the onset of the ragweed pollen season. As can be seen from both of these charts, both the tanned-cell and the BDB methods revealed an increased titer in the sera following injection treatments. The amount of increase varies considerably from patient to patient, and there is no apparent relationship between the titer of antibody originally present and the increase following treatment.

Table I shows the extent of change in titer by the tanned-cell and BDB techniques. With the former method, sixty-four of the sixty-nine patients showed at least a twofold increase in titer, the sera of four patients were the same, and

TABLE I. COMPARATIVE TITERS OF SIXTY-NINE SERA PRETREATMENT AND POSTTREATMENT I

	TANNED-CELL METHOD		BDB METHOD	
TITER CHANGE	NUMBER	PER CENT	NUMBER	PER CENT
Increase at least				
Twofold	64	92.7	58	84
Fourfold	47	68.4	43	62.3
Eightfold	38	55	22	31.8
Same	4	5.8	9	13.0
Decrease	1	1.4	2	2.9

in one patient the titer was decreased. Forty-seven patients demonstrated at least a fourfold increase in titer, and thirty-eight showed at least an eightfold increase. By the BDB method fifty-eight of the sixty-nine patients showed at least a twofold increase and forty-three showed a fourfold increase, whereas only twenty-two showed an eightfold increase.

Even though the absolute titers as determined by each method differ greatly, the relative degree of increase in the serum titers measured by the two methods before and after treatment appears to be similar. A comparison of the relative increase or decrease in titers determined by the BDB and tanned-cell techniques is given in Fig. 3. These results are summarized in Table II.

TABLE II. COMPARATIVE TITERS OF PRETREATMENT AND POSTTREATMENT I* SERA BY BDB AND TANNED-CELL METHODS

BDB AND TANNED-CELL TITERS INCREASED	TANNED-CELL TITERS INCREASED, BDB TITERS SAME OR DECREASED	BDB TITERS INCREASED, TANNED-CELL TITERS SAME OR DECREASED	BOTH TANNED-CELL AND BDB TITERS DECREASED
54	10	4	1

*Sera taken after course of preseasonal hyposensitization therapy but prior to the ragweed pollen season.

Comparison of Titers of Pretreatment Sera and Posttreatment II Sera.—There were fifty-three paired sera for study by both techniques. As can be seen from Table III, the increase in titer from pretreatment to posttreatment II (preseason) was essentially the same as that seen in a comparison of pretreatment titer to preseason sera titer (posttreatment I) (Table I). Hence, we can assume that there was little or no effect on the titer during the pollen season, even though additional treatment was given. These data are borne out in the next series of experiments and hold true for both the BDB and the tanned-cell techniques.

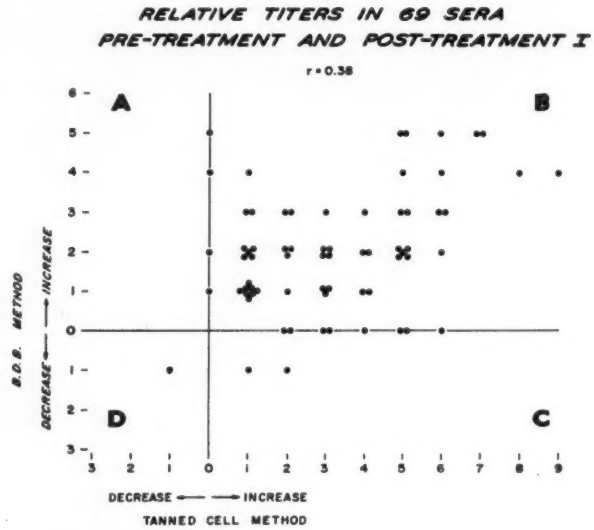

Fig. 3.—*A* indicates an increase in titer by the BDB method and a decrease by the tanned-cell method. *B* shows an increase in titers by both the BDB and the tanned-cell methods. *C* indicates an increase by the tanned-cell method and a decrease by the BDB method. *D* indicates a decrease by both the tanned-cell and the BDB methods. *Horizontal axis:* No change in titer by the BDB method. *Vertical axis:* No change in titer by the tanned-cell method. Each number represents a twofold change. r = Correlation coefficient.

TABLE III. COMPARATIVE TITERS OF FIFTY-THREE SERA PRETREATMENT AND POSTTREATMENT II

TITER CHANGE	TANNED-CELL METHOD		BDB METHOD	
	NUMBER	PER CENT	NUMBER	PER CENT
Increase at least				
Twofold	44	83	41	77.5
Fourfold	34	64.2	30	57
Eightfold	26	49	17	32
Same	7	13.2	5	9.4
Decrease	2	3.8	7	13.2

Comparative Titers of Posttreatment I Sera and Posttreatment II Sera.—The effects of inhalation of ragweed pollen during the pollen season plus additional injection therapy on the hemagglutination titers are illustrated in Figs. 4 and 5. As can be seen from these charts, most of these titers remained relatively the same, and there were just as many that showed an increase as there were that showed a decrease during this time interval (Table IV). The data are further borne out by the fact that of the eight untreated ragweed-sensitive patients, there was no change in titer in two, a decrease in titer in one, and an

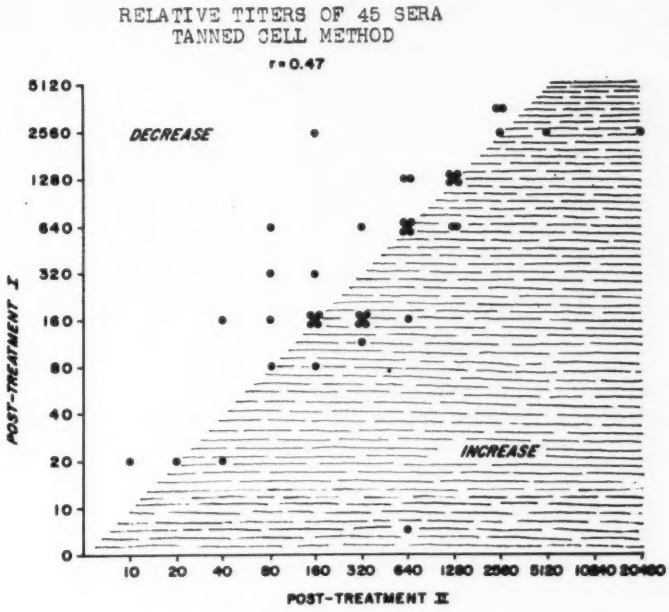

Fig. 4.

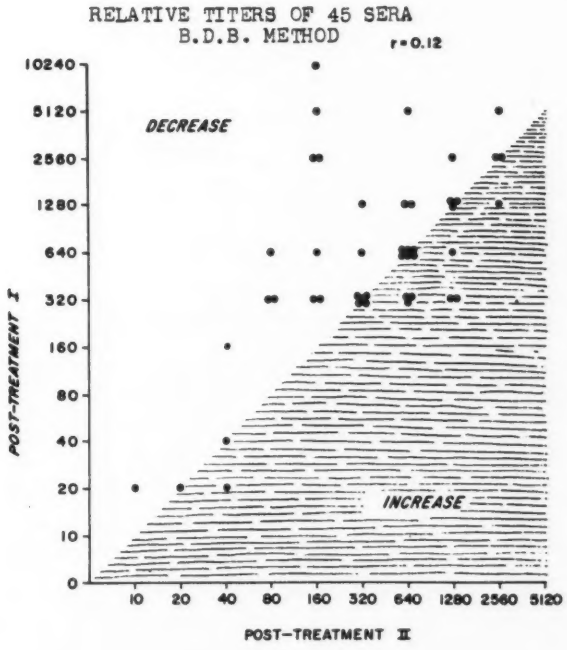

Fig. 5.

Figs. 4 and 5.—*Posttreatment I:* The reciprocal of titers obtained from sera after preseasonal specific hyposensitization therapy prior to the onset of the ragweed pollen season. *Posttreatment II:* The reciprocal of titers obtained from sera after preseasonal specific hyposensitization therapy, additional therapy during the pollen season, and exposure to the inhalation of pollen during the season.

Figures in shaded area show an increase in titer; those above indicate a decrease; and those on the line indicate titers that remain unchanged.

TABLE IV. COMPARATIVE TITERS OF FORTY-FIVE SERA POSTTREATMENT I AND POSTTREATMENT II

TITER CHANGE	TANNED-CELL METHOD		BDB METHOD	
	NUMBER	PER CENT	NUMBER	PER CENT
Increase at least				
Twofold	15	33.3	11	24.4
Fourfold	4	8.9	4	8.9
Eightfold	2	4.5	0	—
Same	21	47	18	40
Decrease	9	20	16	35.6

increase in titer in five by the tanned-cell method, whereas by the BDB method there was no change in titer in three, a decrease in titer in three, and an increase in titer in two (Table V).

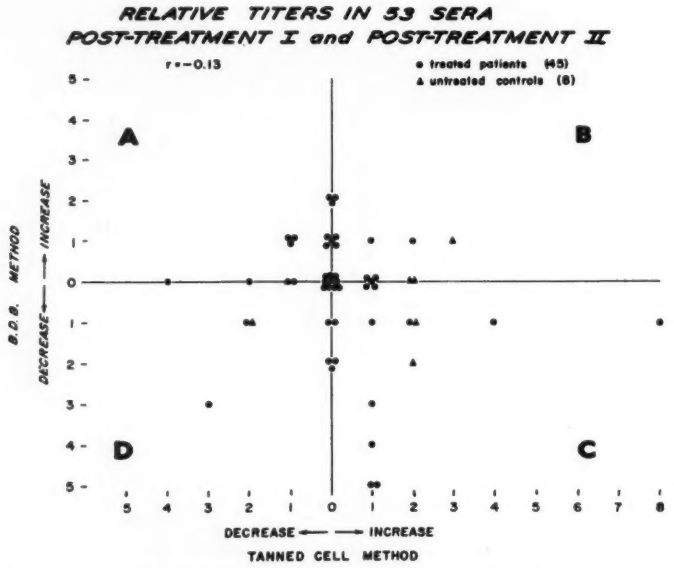

Fig. 6.—*A* indicates a relative increase in BDB titer but a decrease in tanned-cell titer. *B* indicates a relative increase in both BDB and tanned-cell titers. *C* shows a relative increase in tanned-cell titer and a decrease in BDB titer. *D* indicates a decrease in both BDB and tanned-cell titers.

Posttreatment I: Sera obtained after preseasonal specific hyposensitization therapy but prior to the ragweed pollen season. *Posttreatment II:* Sera obtained after preseasonal specific therapy, additional therapy during the pollen season, and inhalation of pollen during the ragweed season.

Horizontal axis: No change in BDB titer. *Vertical axis:* No change in tanned-cell titer.

TABLE V. COMPARATIVE HEMAGGLUTINATION TITERS OF EIGHT SERA (CONTROLS),* PRESEASON TO POSTSEASON

TITER CHANGE	TANNED-CELL METHOD	BDB METHOD
Increase	5	2
Same	2	3
Decrease	1	3

*Untreated ragweed-sensitive patients.

The relative change in titers of sera collected prior to and after the ragweed pollen season (comparison of posttreatment I and posttreatment II) is illustrated in Fig. 6. These results include forty-five treated patients and eight

untreated control patients. Once again, we note that there is no correlation between the two techniques (BDB and tanned-cell), with either the controls or the treated patients, and there was no appreciable change in titers. These results indicate that neither the inhalation of ragweed pollen during the hay fever season nor the additional therapy given during this season, once the patients had already reached their maximum dosage prior to the season, had any effect on the hemagglutination titer.

Effect of Amount of Treatment and Number of Injections on Hemagglutin Titers.—The total amount of extract which each patient received from the start of treatment was recorded. This included all material injected from the pretreatment bleeding until the time the posttreatment I (preseason) specimen was obtained. As can be seen from Table VI, we found no correlation between the amount of treatment that the patient received and the change in hemagglutination titers by either the BDB or the tanned-cell method. These data were carefully evaluated statistically.

TABLE VI. EFFECT OF AMOUNT OF PRESEASONAL SPECIFIC THERAPY ON HEMAGGLUTINATION TITERS

TOTAL AMOUNT OF EXTRACT GIVEN*	NUMBER OF PATIENTS	HEMAGGLUTINATION TITER CHANGE					
		BDB METHOD			TANNED-CELL METHOD		
		INCREASE	SAME	DECREASE	INCREASE	SAME	DECREASE
25	2	1	1		2		
25 to 2500	23	20	1	2	21	2	
2500 to 10,000	19	16	3		17	2	
10,000 to 25,000	12	10	2		11	1	
25,000	13	12	1		13		

*Total amount of short ragweed pollen extract expressed in protein nitrogen units (P.T.A./n) given from pretreatment bleeding time to posttreatment I (preseasonal) specimen was collected.

Similar studies comparing the amount of treatment received during the pollen season and the posttreatment I and II hemagglutination titers likewise showed no correlation. Also, no relationship between the total amount of preseasonal and seasonal treatment and the preseason and posttreatment II titers was revealed by either method.

Sixty-nine patients were followed with pretreatment and posttreatment I sera. They received anywhere from a minimum of four injections to a maximum of thirty-two. Here again, there was no correlation between the number of injections of ragweed pollen extract and the change in hemagglutination titers as analyzed by our statistician.

DISCUSSION

In all but four of the sixty-nine patients studied, there was some increase in tanned-cell titer following injection treatments with pollen extracts. Inasmuch as the titers of the various samples of a particular patient's serum were determined at the same time, it would seem that even a twofold difference would be important. As with all serologic tests, however, if one were to consider only a fourfold or more difference as significant, there still is a high percentage (66 per cent) of increases in titer following treatment. If one were even more

critical and considered an eightfold or more increase in titer as significant, then over 50 per cent of the patients had such an increase by the tanned-cell technique.

Comparison of sera collected prior to treatment and at the end of treatment after the season had been completed showed increases similar to those of the pretreatment to the posttreatment I sera. This would indicate that the inhalation of ragweed pollen during the hay fever season and additional treatment which had been given, once the maintenance dose had been reached, did not significantly alter the hemagglutination titers. This fact is borne out very well when one compares the sera of the posttreatment I (preseason) patients to the sera of the posttreatment II (postseason) group of patients. For the most part, there was no significant change in these titers. Of those that did change, the number that increased was essentially the same as the number that showed a decrease in titers by either of these techniques. Likewise, among the eight patients who were ragweed sensitive and did not receive any injection treatments, apparently there was no significant change in the hemagglutination titers during their exposure to ragweed pollen inhalation. As a matter of fact, by the BDB method, in several instances there was a considerable decrease in the hemagglutination titer during the ragweed pollen season. This possibly could be due to a neutralization of antibodies produced by the inhalation of the ragweed antigen.

There was likewise no relationship between the amount of antibody present prior to treatment and the relative increase following the injection therapy. Apparently, there is a maximum increase, as evidenced by the fact that additional treatments during the pollen season did not materially affect an increased hemagglutination titer.

All of these data were subjected to analysis by statisticians. The only definite correlation which we could find with these various comparisons was in the experiments comparing the relative change in titers by both the BDB and the tanned-cell techniques. Here the correlation coefficient was 0.38. This would indicate that the amount of increase revealed by both methods is correlated.

Although we could find no correlation between the two techniques, the results indicated that there is an increase in hemagglutination titers after specific injection therapy and that the inhalation of ragweed poller does not materially affect this change in hemagglutination titer.

The relationship of the increase in the hemagglutinating antibody titer to the changes in reagin titer, as well as results of therapy, will be the subject of our next report. The question naturally arises: "What are we measuring? Are we measuring the reagin content of the sera, the blocking antibody, or some other antibody or antibodies increasing as a result of injection therapy?"

SUMMARY AND CONCLUSIONS

Sera from ragweed-sensitive patients were obtained prior to and after injection treatments with specific ragweed pollen antigen. The hemagglutination

titers by the BDB and tanned-cell methods were determined, and the following conclusions can be reached:

1. There is an increase in hemagglutination titer following specific hyposensitization therapy.

2. There is no observable effect on the hemagglutination titer by the inhalation of ragweed pollen during the hay fever season.

3. The total amount of treatment and the number of injections of ragweed pollen extract had no direct effect on the change in hemagglutination titer.

We wish to thank Mrs. Hazel Popp and June Spohr for their technical assistance, Dr. Edward Dowd of the Roswell Park Memorial Institute for his statistical evaluation of the data presented, and Mrs. W. P. Stafford, Jr., for her financial assistance.

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IMMUNOLOGIC STUDIES OF RAGWEED-SENSITIVE PATIENTS

III. Clinical Aspects: The Relationship of Reagin and Hemagglutinating Antibody Titers to Results of Hyposensitization Therapy

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THE first two articles of this series[1-2] demonstrated that about 90 per cent of the sera from ragweed-sensitive patients had positive hemagglutination tests against ragweed and that in at least two-thirds of the patients the titer of this antibody increased fourfold or more following specific hyposensitization therapy. It was also shown that there was no relationship between the age of the patient, duration of previous symptoms, amount of treatment given, or number of injections and the increase in hemagglutination titer.

The purpose of this article is to demonstrate the relationship of the change in hemagglutination titer to the change in titer of skin-sensitizing antibody (reagin) and the degree of relief from specific hyposensitization therapy. In addition, the effect of the amount of pollen extract injected and the number of injections on the change in reagin titer and the degree of relief obtained will be reported.

Gordon and associates[3] have shown that there is a close relationship between the hemagglutinating and reagin titers. Others[4] have reported that there is an initial rise in reagin titer following specific hyposensitization therapy and then a return to the same or lower levels with continued treatment. There has been no evidence of actual desensitization (disappearance of reagin) following intensive specific hyposensitization pollen therapy, and no relationship has been demonstrated between the change in reagin titer and the results of specific therapy.[5]

METHODS AND MATERIALS

The hemagglutination titers were determined by both the tanned-cell and the bis-diazotized benzidine methods previously described.[1]

Reagin Titers.—At least two, and sometimes three, samples of serum from each of twenty-seven patients were studied. Serial twofold dilutions in normal

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saline of the pretreatment and posttreatment sera were injected into each of two nonallergic recipients (skin-test negative to ragweed). These sites were marked, and twenty-four to forty-eight hours later each site was tested with a constant amount of ragweed pollen extract (1,000 U. PTAN strength). Fifteen minutes after the challenging injections, the degree of wheal and erythema for each site was recorded. The highest dilution of serum that gave a definite positive reaction was called the reagin titer.

Results of Treatment.—Treatment results were arbitrarily classified as excellent, good, fair, or poor on the basis of the patient's subjective evaluation and the investigators' objective findings. Determining factors included physical examination of the nasal mucosa, the number of complaints, and the amount of symptomatic medication taken.

RESULTS

*Comparison of Reagin and Hemagglutination Titers of Twenty-seven Pretreatment Sera.**—The titers of both the hemagglutinating and the passive transfer (reagin) antibodies are graphically shown in Figs. 1 and 2. Fig. 1 demonstrates the lack of correlation between the reagin titer and the hemagglutination titer as determined by the tanned-cell method. It can be seen that six of the twenty-seven sera tested had no demonstrable hemagglutination titer but did have reagin titers. Moreover, one serum had a high hemagglutination titer but a relatively low reagin titer (320 to 8, respectively). Contrawise, three sera had reagin titers of 512 or greater and hemagglutination titers of 1 to 10 or less.

Fig. 2 shows the same lack of correlation between reagin titer and hemagglutination titer as determined by the bis-diazotized-benzidine (BDB) method.

Comparison of Relative Change in Reagin and Hemagglutination Titers Following Specific Hyposensitization Therapy.—The actual change in titers by twofold dilutions is shown in Figs. 3 and 4. The reagin titer remained unchanged in fifteen of the twenty-seven sera tested, it was decreased in one, and it was increased in eleven. As will be noted, however, sera from all but one of the patients showed an increase in hemagglutination titer by the tanned-cell method. Similar results were obtained when the BDB method of hemagglutination was used (Fig. 4). Two sera showed a decrease in BDB hemagglutination titer. In one there was no change in reagin titer, whereas the other showed a fourfold increase. These results are summarized in Table I.

TABLE I. COMPARISON OF RELATIVE CHANGE IN HEMAGGLUTINATION AND REAGIN TITERS FOLLOWING SPECIFIC HYPOSENSITIZATION THERAPY

TECHNIQUE	NO. OF SERA WITH INCREASED HEMAGGLUTINATION TITER	CHANGE IN REAGIN TITER		
		INCREASE	SAME	DECREASE
Bis-diazotized-benzidine	23	8	14	1
Tanned-cell	26	11	14	1

*These sera were obtained from five patients who never received any specific hyposensitization therapy with ragweed pollen extract and twenty-two patients who had undergone treatment in previous years but had received no treatment for at least one year.

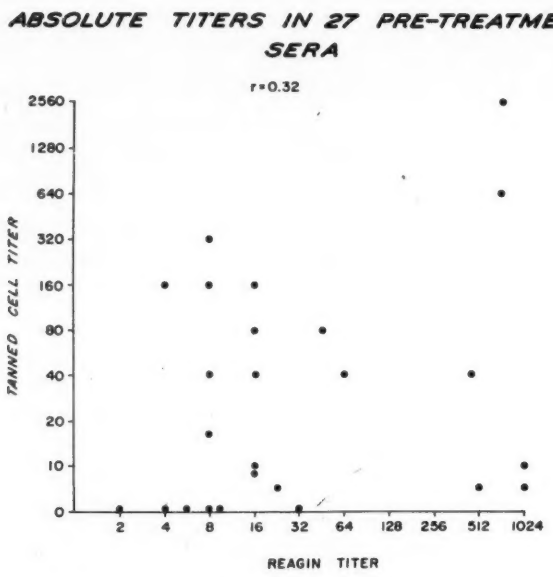

Fig. 1.—Black dots represent the tanned-cell titer and the reagin titer of each patient.

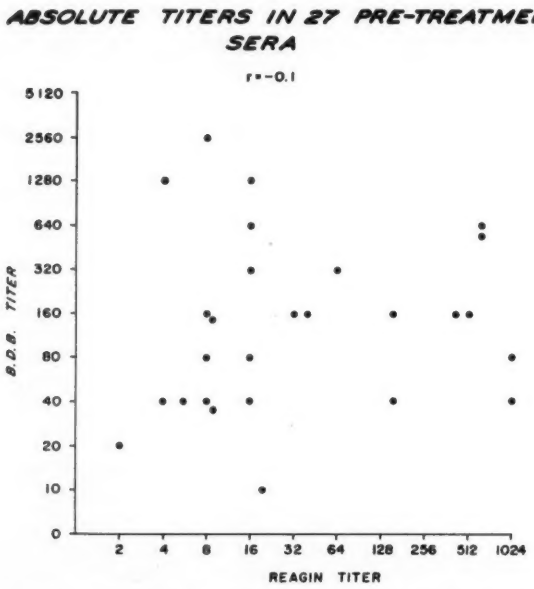

Fig. 2.—Black dots represent the BDB titer and the reagin titer of each patient.

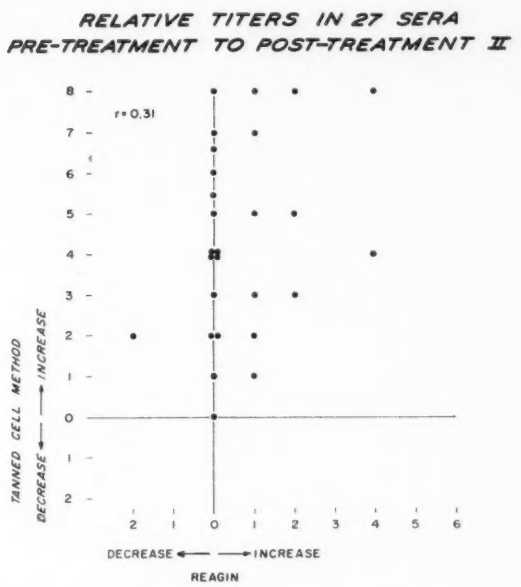

Fig. 3.—Comparison of the relative increase or decrease in tanned-cell titer with the change in reagin titer from the beginning to the end of the observation period. The numbers indicate the number of twofold dilutions increased or decreased.

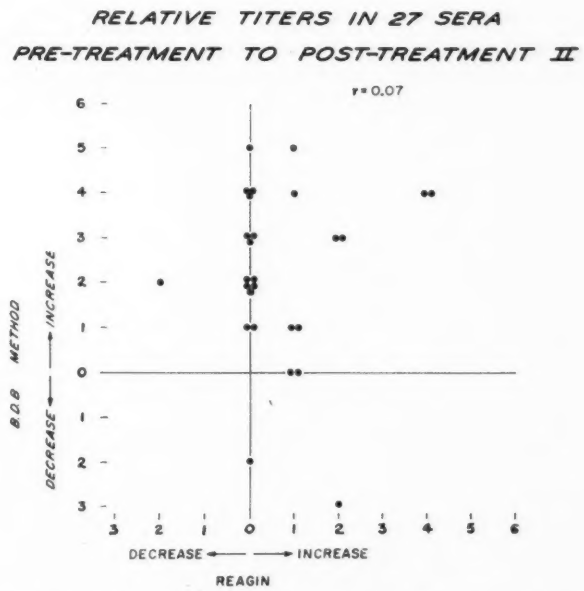

Fig. 4.—Same comparison as shown in Fig. 3, but based on the BDB instead of the tanned-cell test.

Changes in Reagin Titer During Course of Hyposensitization Therapy.—Three samples of sera from ten of the twenty-seven patients were studied for their skin-sensitizing activity. All three samples of four of these sera showed no change in reagin titer. There was a decrease in titer in one patient's post-treatment I serum,* but then a marked increase in titer over the pretreatment serum was seen in the posttreatment II serum.† There was a steady increase in the reagin titer with each successive sample during hyposensitization therapy in two patients. In the remaining three patients the reagin titer was unchanged between the first and second samples of sera, but there was an increase in titer with continued therapy in the third (posttreatment) sample.

Effect of Amount of Treatment and Number of Injections on Change in Reagin Titer.—There appeared to be no relationship between the total amount of pollen extract given a patient and the change in reagin titer. In some patients who received relatively large dosages of pollen extract there was no change in reagin titer, whereas in others who received much smaller dosages there was an increase in titer. Likewise, the total number of injections of pollen extract had no relationship to the change in reaginic titer. The titer changes, the total amount of treatment, and the number of injections are shown in Figs. 5 and 6. One can readily see that in some cases the reagin titer did not change, even when as much as 180,000 protein nitrogen units of ragweed extract was given, whereas the patient with a twelvefold increase in reagin titer received a total of only 10,000 protein nitrogen units. As to the number of injections of extract influencing the reagin titer, Fig. 6 shows that of three patients receiving twenty-four injections, one patient had no change in reagin titer, another had a twofold increase, and the third had a twelvefold increase.

Effect of Amount of Treatment and Number of injections on Results of Hyposensitization Therapy.—Seventy-four patients received specific hyposensitization therapy with ragweed pollen extract. Of this group, eighteen had excellent relief and thirty-five had satisfactory results. Those who had good or excellent results received much larger amounts of pollen extract than those who gave only a fair or poor response to treatment (Table II).

TABLE II. RESULTS OF TREATMENT

RESPONSE TO THERAPY	NUMBER OF PATIENTS	AVERAGE TOTAL AMOUNT OF EXTRACT*
Excellent	18	38,100
Good	35	34,200
Fair	8	13,500
Poor	13	7,200
Total	74	

*Expressed in PTA nitrogen units.

There is no apparent correlation between the results of treatment and the number of injections used for specific hyposensitization (Table III). However,

*Posttreatment serum but previous to ragweed season.
†Posttreatment serum but following ragweed season.

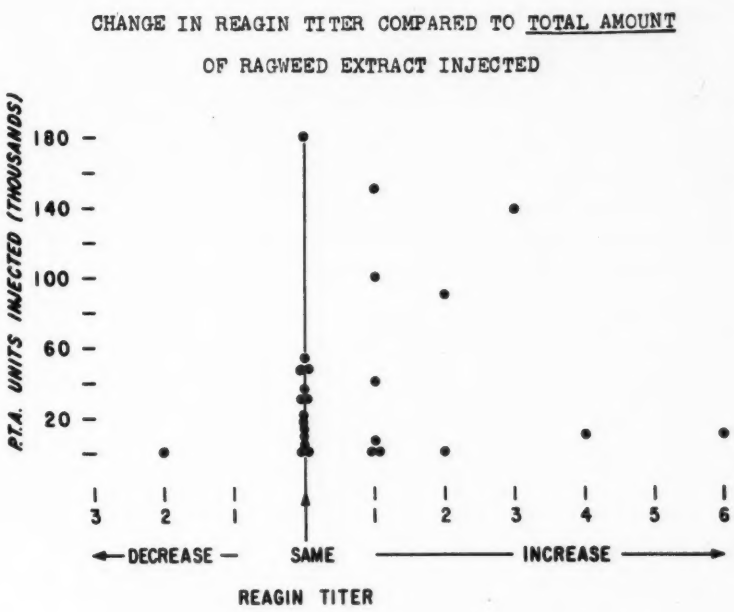

Fig. 5.

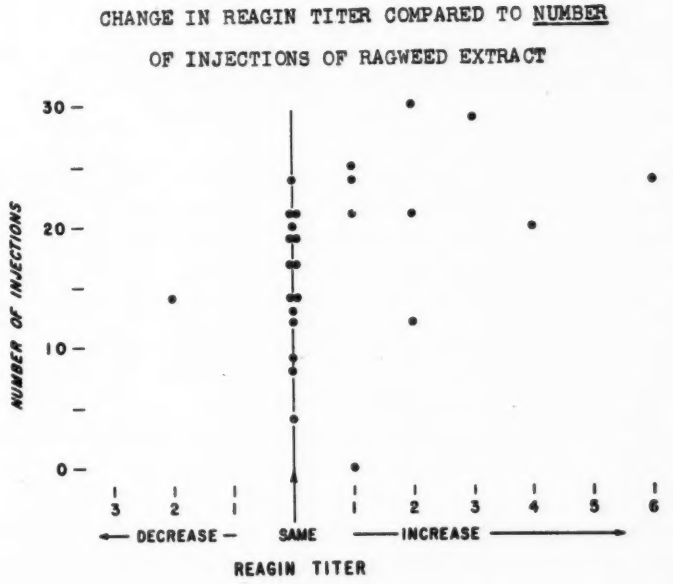

Fig. 6.

Figs. 5 and 6.—The change of reagin titer is expressed by the number of twofold dilutions increased or decreased during the observation period.

those patients who had the best results of treatment received, on the average, more injections than any other group.

TABLE III. RESULTS OF TREATMENT

RESPONSE TO THERAPY	NUMBER OF PATIENTS	AVERAGE NUMBER OF INJECTIONS
Excellent	17	20.4
Good	31	14.2
Fair	8	17.8
Poor	12	15.1
Total	68	

Response to Specific Hyposensitization Therapy and Change in Hemagglutination Titer.—Response to therapy was excellent in seventeen of the sixty-nine patients studied and good in thirty-two. The mean change in hemagglutination titer (increase), determined by both the BDB and tanned-cell methods, following pollen injection therapy is shown in Table IV. Although it appears that those patients with a satisfactory response to therapy (good or excellent) had a greater mean change in titer than those with a poor response, the difference is not statistically significant.

TABLE IV. RESULTS OF TREATMENT

RESPONSE TO THERAPY	NUMBER OF PATIENTS	MEAN CHANGE IN TITER	
		BDB	TANNED-CELL
Excellent	17	2.3	2.64
Good	32	2.0	3.6
Fair	8	2.0	2.0
Poor	12	1.4	2.4

DISCUSSION

Although an increase in the hemagglutination antibody following specific hyposensitization has been observed, its significance is as yet unknown. We found no relationship between the results of therapy and changes in these hemagglutination titers. This may or may not indicate that we are measuring some type of blocking or protective antibody, as others[6, 7] have shown that there is not always a correlation between the blocking antibody titer and the clinical response. Experiments are now in progress to compare the so-called blocking antibody titer to the hemagglutinating antibody titer.

In our experiments, we found no correlation in either the actual reagin and hemagglutination titer or the relative changes in each following treatment. This would indicate that we are not simply measuring the skin-sensitizing antibody by these techniques. In addition to this fact, all the sera tested for hemagglutination were inactivated and titers were not affected, whereas it is well known that reagin is inactivated when heated to 56 degrees for one hour.

In only one patient did we note an initial fall in reagin titer followed by a rise on continued therapy. This does not confirm the work reported by Sherman.[4]

Of the twenty-seven groups of sera tested, only eleven showed an increase in reagin titer, whereas fifteen remained unchanged and one demonstrated a decrease. One might argue that this method of determining reagin is not very reproducible. By using two recipients for each set of sera, however, it is possible to meet some of those objections.

Neither the amount of pollen extract given nor the total number of injections had any effect on the change in reagin titer. This same lack of relationship to the changes in the hemagglutinating antibody has already been reported[2] in a previous publication. It appears, however, that those patients who received or could tolerate the larger amounts of pollen extracts had a better therapeutic response.

The results of our three articles and the reports of others clearly show that the sera of most patients suffering from ragweed hay fever contain antibodies against ragweed which are demonstrable by indirect hemagglutination methods. In general, experience indicates that these tests measure precipitating antibody but are at least 1,000 times more sensitive than ordinary precipitin tests. We must consider the additional possibility that nonprecipitating or "incomplete" antibody may also play a role in the hemagglutination reactions, although the evidence for this is not entirely clear.

Evaluation of the significance of this hemagglutination antibody (or these antibodies) is rather difficult at this time. At first observation, one may think that the BDB reaction measures only a nonspecific serum protein that has agglutinating properties. Such a protein may indeed account for some of the agglutination observed by this method, at least in low dilutions of all human sera. On the other hand, we recognize that the reaction is quite specific for ragweed and does not even extend to the serologically related antigen, June grass. Furthermore, the titer of hemagglutination increases significantly during injection of ragweed extract. For this reason, we regard the BDB reaction as due, at least partly, to an antibody against ragweed. This antibody is present in both normal and allergic persons, conceivably due to exposure to ragweed or to a related antigen in the environment. The antibodies are generally stronger in persons allergic to ragweed and occur in a greater percentage of this group than in the general population.

The tanned-cell hemagglutination test also appears to measure a specific antibody to ragweed. This antibody is found predominantly in persons allergic to ragweed. It is not impossible that the approximately 15 per cent of "normal" persons showing reactions by this method have some clinical or subclinical allergy to ragweed. Thus, it would appear that this antibody is rather characteristic of ragweed-sensitive persons. The reason for this correlation is not known; certainly, the hemagglutinating antibodies themselves do not directly produce the symptoms of the disease, since they are not related to the severity or duration of symptoms. Perhaps their presence in the sera of allergic persons signifies a greater immunologic responsiveness of ragweed-sensitive patients to this antigen, embracing precipitating as well as skin-sensitizing varieties of antibodies.

Both forms of the hemagglutination test agree in revealing significant increases in antibody titer following hyposensitization. Essentially, this finding indicates that ragweed is antigenic in man, as it is in rabbits. In direct contrast, reagins are not consistently affected by these treatments, and not infrequently they actually decrease during hyposensitization. This finding, together with the difference in heat stability of the two antibody functions as previously described, leads us to believe that reagin does not play any significant role in the hemagglutination reactions. It is impossible, as Gordon and colleagues[3] have pointed out, to exclude its participation as one of the antibodies reacting in the hemagglutination tests, but there is certainly no positive evidence of its contribution to the reaction. On the contrary, some patients with skin-sensitizing antibody had no demonstrable hemagglutination reaction. We could also find no direct relationship between the benefits of treatment and the increase in hemagglutination titer. If one chooses to attribute the effects of hyposensitization to the production of "blocking antibodies," it must again be supposed that the blocking antibody may represent only one of several antibodies producing hemagglutination. Until purified fractions of ragweed are available for this sort of testing, overemphasis on the lack of correlation will be premature.

SUMMARY AND CONCLUSIONS

1. There was no relationship between either the absolute titer or the change in hemagglutination and reagin titers following specific hyposensitization therapy.

2. There was no relationship in the clinical response to either the change in hemagglutination or reagin titers or the number of injections of pollen extract.

3. The best response to hyposensitization therapy was seen in those patients who received the largest total amount of pollen extract.

We wish to thank Dr. Edward Dowd of the Roswell Park Memorial Institute for his statistical evaluation of the data.

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INVESTIGATIONS OF SPONTANEOUS HYPERSENSITIVITY OF THE DOG

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SPORADIC reports of spontaneous hypersensitivity in animals have appeared periodically in the veterinary and medical literature.[1-5] The objectives of the investigations reported here were (1) to study a case of spontaneous ragweed pollen sensitivity in the dog, using some of the clinical and immunologic procedures presently available, (2) to determine the usefulness of such an animal as a model of allergy in investigative studies, and (3) to evaluate the effect of certain pharmacologic materials and other factors on a reproducible quantitative allergic response of the animal.

CASE REPORT

A 2-year-old ovariectomized fox terrier living in southern Michigan was first noted to develop symptoms of lacrimation, conjunctivitis, and severe pruritus in mid-August, 1957, coincident with the pollination of ragweed. Her condition was diagnosed as an allergic reaction, and she was treated with antihistamines with some relief of symptoms. The symptoms subsided at the end of the ragweed season in early October, 1957, and she was next seen in mid-August, 1958, with the return of the same symptoms. J. A. Hergott, D.V.M., then made the dog available to us for investigation.

Physical examination showed the dog to be in good general health. Positive physical findings included reddening of the conjunctivae, profuse lacrimation, and some purulent discharge from the eyes. An erythematous, scaling eruption was present over the back and on the flexural surfaces of the forelegs. The remainder of the physical examination was negative. The symptoms persisted through August and September and began to subside in early October. The ocular symptoms cleared completely by the middle of October. The skin lesions improved but persisted until the middle of December, when they gradually subsided.

Experimental Data.—The total white blood cell count was 13,000, with a differential count of 72 polymorphonuclear leukocytes, 13 lymphocytes, 5 monocytes, and 10 eosinophils. A conjunctival smear stained with eosin and methylene blue showed large clumps of polymorphonuclear leukocytes. Intradermal skin tests with 1:500 concentrations of pollen, mold, and dust extracts were done, using saline as a control. All skin tests were negative, with the exception of those to ragweed, which showed a 3 plus reaction. Serum was obtained from the dog by a sterile technique, and 0.05 ml. of this serum was planted in normal human skin and normal dog skin. In forty-eight hours these skin sites were challenged with 1:500 ragweed antigen, and a 4 plus reaction was observed in both human skin and dog skin with

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negative controls. Conjunctival testing with ragweed pollen was positive, but testing with grass and tree pollen was negative. Similar tests were negative in the normal dog used as a control. Attempts to demonstrate precipitating antibodies with ragweed antigen and dog serum in routine precipitin tests and with the Ouchterlony double-diffusion gel-precipitin technique[6] were negative. As expected, human antiragweed serum used in identical techniques was negative and rabbit antiragweed serum prepared by hyperimmunization of rabbits with ragweed antigen in Freund's adjuvant was positive. Active cutaneous anaphylaxis by the method of Ovary[7] was positive in the dog skin. For this test, 0.02 ml. of 1:500 ragweed antigen was injected intradermally and was immediately followed by the intravenous injection of 1 ml. of 0.5 per cent Evans blue dye. A positive reaction was noted, with a dark blue area at the site of the antigen injection, and the saline control was negative. Passive cutaneous anaphylaxis was attempted, using the method of Ovary.[7] Separate skin sites in

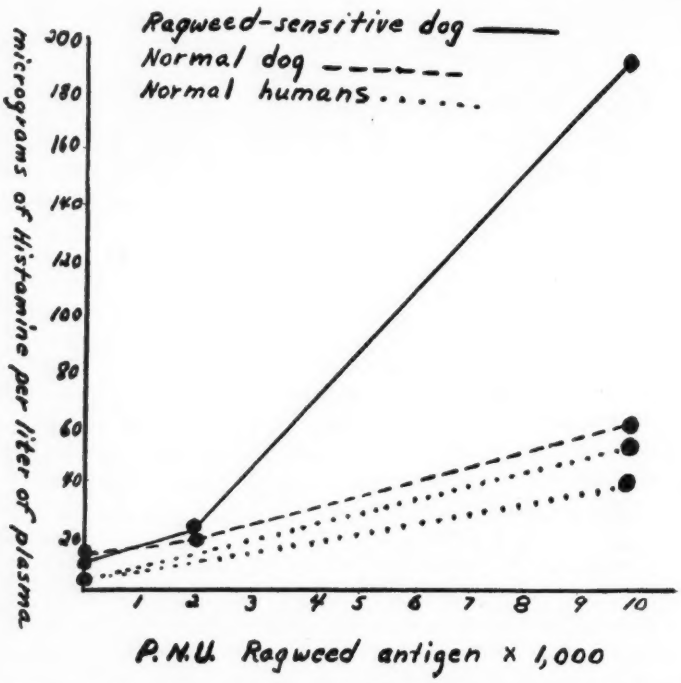

Fig. 1.—Release of histamine from blood of ragweed-sensitive dog and normal controls after addition of ragweed antigen.

the normal white guinea pig were sensitized intradermally with 0.05 ml. of dog antiragweed serum and 0.05 ml. of human antiragweed serum. Twenty-four hours later these skin sites were challenged by intradermal injection of ragweed antigen and intracardiac injection of Evans blue dye. Positive Prausnitz-Küstner reactions were noted, but the passive cutaneous reaction was negative. Similar results using human serum alone have been described by Fisher and associates.[14] A bis-diazotized benzidine hemagglutination reaction, using a modification of the method described by Gordon and associates,[15] was done* and was positive at a titer of 1:1,280.

The release of histamine after the addition of ragweed antigen to blood from the ragweed-sensitive dog and normal controls was measured* as described by Noah and Brand,[8] and results are shown in Fig. 1.

A summary of the findings in the ragweed-sensitive dog and a comparison with findings in human ragweed sensitivity is shown in Table I.

*Performed by Dr. K. P. Mathews.

TABLE I. COMPARISON OF RESULTS OF CLINICAL AND IMMUNOLOGIC STUDIES DONE ON THE RAGWEED-SENSITIVE DOG AND ON RAGWEED-SENSITIVE HUMAN BEINGS

TEST	RAGWEED-SENSITIVE HUMAN BEINGS	RAGWEED-SENSITIVE DOG
Skin testing with ragweed antigen	Positive	Positive
Conjunctival testing with ragweed pollen	Positive	Positive
Passive transfer of reaction to normal skin with serum (Prausnitz-Küstner reaction)	Positive	Positive
Precipitin reaction with serum and ragweed antigen (routine precipitin and Ouchterlony plate technique)	Negative	Negative
Active cutaneous anaphylaxis	Not done	Positive
Passive cutaneous anaphylaxis in guinea pig skin using serum from ragweed-sensitive patient	Negative	Negative
Bis-diazotized benzidine hemagglutination titer	Positive (some)	Positive
Symptoms produced by exposure to ragweed antigen	Conjunctivitis, rhinitis, respiratory distress	Conjunctivitis, rhinorrhea, respiratory distress
Symptoms relieved by antihistamines, epinephrine, and adrenal cortical hormones	Yes	Yes
Histamine release from blood after addition of ragweed antigen	Usually	Suggestive

DETERMINATION OF A QUANTITATIVE RESPONSE TO RAGWEED POLLEN

In order to determine the value of the dog as an experimental animal in allergy investigation, it appeared necessary to find which allergic symptom was reproducible and could be measured by either time of onset or severity of response. After exposure to small amounts of ragweed pollen or ragweed antigen in an animal chamber, rhinorrhea, lacrimation, and conjunctivitis developed, but these symptoms were variable in time of onset and degree and eluded any quantitative evaluation. It was then found that exposure to a sufficient quantity of aerosolized pollen or ragweed pollen extract in an animal chamber resulted in a striking symptom complex of severe dyspnea characterized by increased frequency of respirations, thoracic respirations instead of the usual abdominal type, use of accessory muscles of respiration, an audible wheeze, prolonged expiratory phase of respiration, increased expansion of the thorax (an expansion of approximately 2 cm. or 4 per cent of the circumference of the thorax in the normal resting state), and assumption of a sitting or standing position. Auscultation of the chest during an attack demonstrated sonorous musical râles throughout the chest, which were more marked during the prolonged expiratory phase. During the acute attack, the dog refused to eat even her favorite foods, and if the attack was allowed to persist she developed vomiting and diarrhea. The attack of dyspnea would gradually subside over a period of approximately ninety minutes after antigen administration was discontinued or in one-third that time after the subcutaneous administration of 0.3 ml. of 1:1,000 aqueous epinphrine. This attack was then considered to be an asthmatic attack in the ragweed-sensitive dog due to ragweed pollen. It was found that the attack was reproducible, provided that sufficient antigen was delivered to the animal in the chamber, and that no reaction occurred with other pollen antigens, including grass and tree pollen. Normal dogs exposed to ragweed antigen under identical circumstances did not have reactions. This response of

the animal was developed as the method of study of a quantitative allergic response because of its consistent reproducibility and the striking end point. The apparatus designed for the experiments is shown in Fig. 2. The dog is placed in the animal chamber with the pneumograph belt in place and allowed to reach a resting state. The dog is allowed freedom of movement by flexible rubber-tubing attachments. Temperature in the chamber is kept constant at 25° C. by a cooling area in the sub-flooring. Undefatted pollen is delivered into the

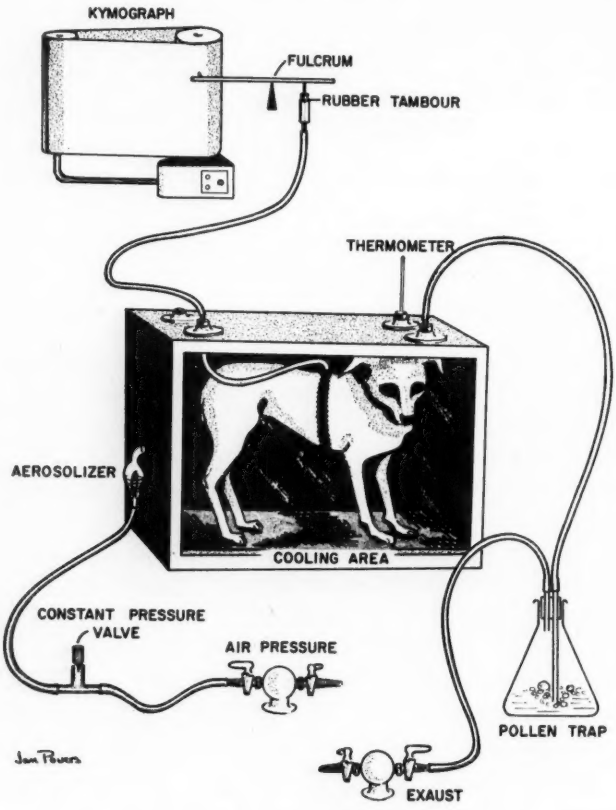

Fig. 2.—Apparatus used for exposure to pollen in animal chamber. Considerable freedom of movement is allowed by the size of the chamber.

chamber through a DeVilbiss No. 40 nebulizer. A constant amount of pollen is delivered per minute by means of a constant pressure valve in the air pressure line leading to the nebulizer. This results in a constant flow of the pollen suspension from the nebulizer. Undefatted pollen is suspended in distilled water by thorough mixing. A pollen-in-water suspension is used, since dry pollen alone does not flow through the nebulizer evenly and cannot be counted as accurately as the pollen-in-water suspension. The concentration of pollen is determined by counting in a hemocytometer chamber, using a white blood cell pipette and the technique used for counting white blood cells. Suitable

dilutions are made in order to deliver varying concentrations of ragweed pollen into the chamber. The animal chamber is sealed to prevent laboratory contamination with pollen, and air is evacuated from the chamber at a rate equal to the inflow through the exhaust leading to the pollen trap. Humidity in the chamber is constant during each experiment because of the constant flow of pollen-in-water suspension. In order to record the asthmatic attack graphically, a kymograph record using either smoked paper or an ink-writing apparatus was used.

Development of the typical asthmatic attack is shown by the tracing in Fig. 3 and is characterized by an increase in rate and amplitude of recorded respirations. Inspiration is written as an upward deflection. The increase in amplitude of the recorded respirations is primarily a reflection of the change from abdominal respiration to thoracic respiration and is *not* an indication of the amount of air inspired as compared with the normal resting state. The record of an experiment in which asthma did not develop is shown in Fig. 4. The subsidence of an attack after cessation of pollen administration is illustrated in Fig. 5, and the more rapid improvement after treatment with epinephrine is shown in Fig. 6. The prolonged expiratory phase is illustrated by increasing kymograph drum speed, as shown in Fig. 7. The decrease in the range of thoracic expansion is illustrated in Fig. 8, *B* and *C*, where the amplitude of deflection during the sniffing of food in Fig. 8, *B* is greater than that during the dyspnea in an asthmatic attack in Fig. 8, *C* and is considered to be a reflection of the acute emphysema and decrease in vital capacity during asthma. Respirations of the panting dog recorded in Fig. 8, *A* are not confused with the typical attack because of the irregularity of the panting hyperventilation, the cessation of this hyperventilation with cooling of the chamber, and the lack of other clinical manifestations described above. To avoid confusion, however, the dog was always allowed to reach a resting state prior to administration of antigen. The degree of amplitude of deflection does not reflect severity of attack and varies in different records, depending on positioning of the pneumograph belt on the dog's thorax and technical variations in the recording apparatus.

Following the establishment of an asthmatic response which was reproducible and possible to record, it was next necessary to develop a method by which the minimal amount of antigen necessary to cause an attack could be determined so that observations on alteration of the dose-response relationship could be undertaken. In all subsequent experiments, commercial undefatted *Ambrosia elatior* pollen was used. After a concentration of pollen which would produce asthma had been arbitrarily chosen, smaller but increasing amounts of pollen were administered at daily intervals to find the minimal concentration of pollen which would produce the symptoms. It was then found that the daily administration of pollen to the dog resulted in the development of a state of tolerance to a dose which previously had successfully produced an asthmatic attack. Furthermore, by increasing the pollen concentration delivered during the same experiment after a definite period of time when no asthma occurred, it was found that a certain concentration of pollen was necessary to produce an

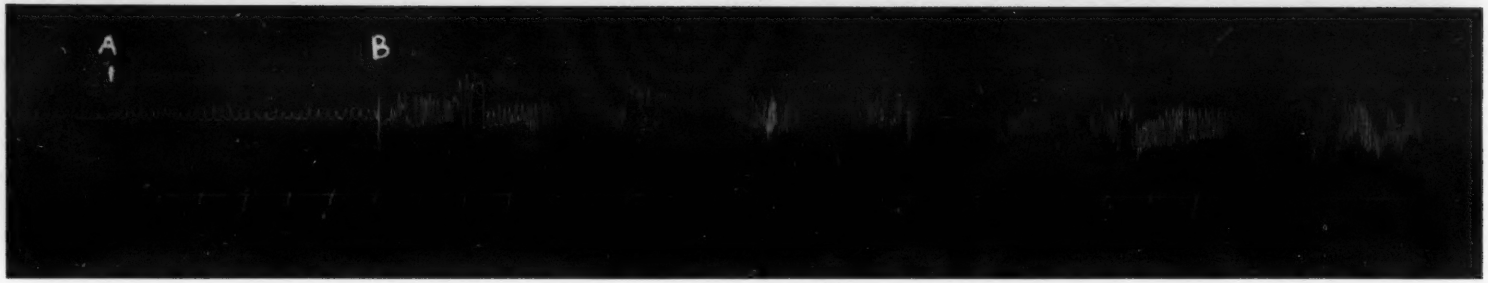

Fig. 3.—Development of asthmatic attack in the dog. *A*, Delivery of ragweed pollen suspension begun. *B*, Recording drum stopped for period of eight minutes. The lower tracing records time intervals of ten seconds.

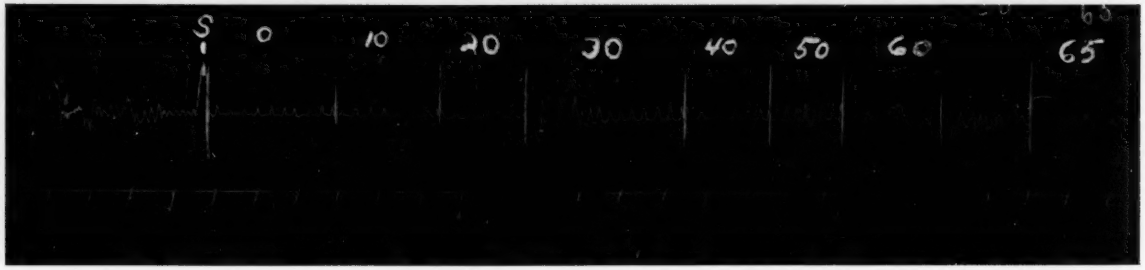

Fig. 4.—Record of respirations during sixty-five minutes of aerosolization of 1.5 per cent serotonin creatinine sulfate. *S*, Initiation of serotonin. Short recordings made every five minutes.

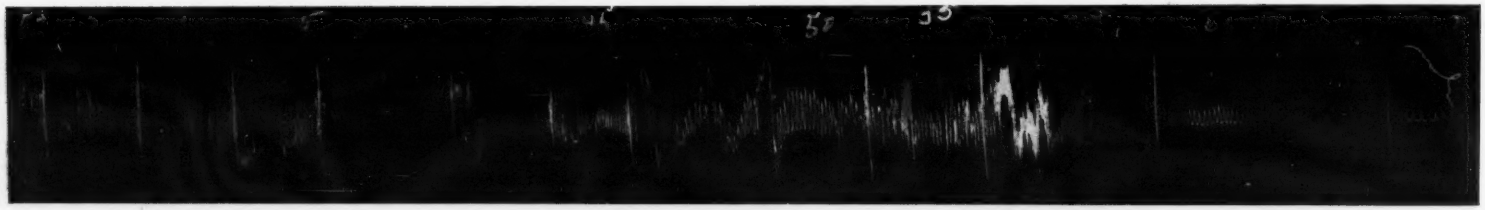

Fig. 5.—Subsidence of asthmatic attack after cessation of ragweed pollen aerosolization. *PS*, Pollen stopped. Short recordings taken every five minutes for eighty minutes. Kymograph drum speed the same as in Fig. 3.

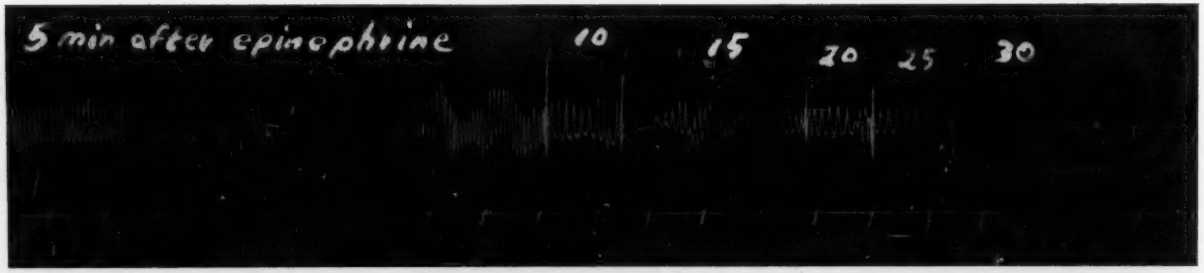

Fig. 6.—Subsidence of asthmatic attack after administration of 0.3 ml. of 1:1,000 aqueous epinephrine subcutaneously. Short recordings at five-minute intervals.

attack of asthma and that below this *threshold* concentration of pollen-in-water suspension no asthmatic attack occurred, even though the pollen was continuously delivered for periods up to three hours. When any concentration of the pollen-in-water suspension above the threshold was delivered, the asthmatic attack occurred within ten minutes. In repeated experiments it was found that this threshold phenomenon was reproducible but that the threshold of concentration varied, depending on the period of time since the previous pollen administration. Daily attacks of asthma or subthreshold concentrations of pollen resulted in an increase of the threshold necessary to produce an attack on the following day.

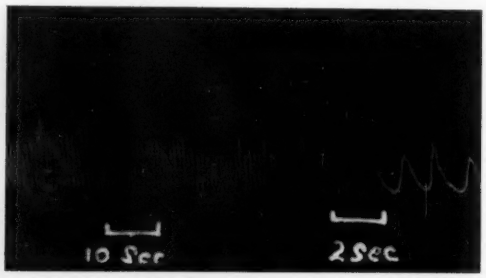

Fig. 7.—Prolonged expiratory phase during asthmatic attack demonstrated by increasing kymograph drum speed.

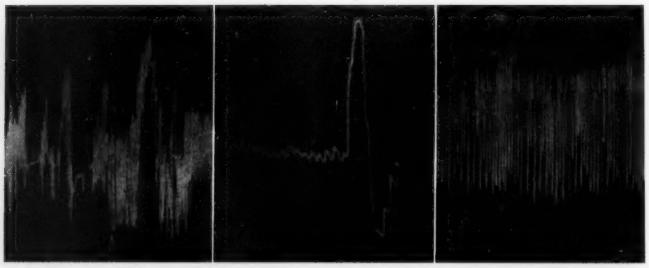

Fig. 8.—Three tracings during one experiment. *A*, Panting respirations following exercise and before pollen administration. *B*, Resting state followed by sniffing food. *C*, Subsequent asthmatic attack which followed pollen administration. Recording speed the same as in Fig. 3.

After a "rest" period of several days without pollen exposure, the threshold dropped to a lower level of concentration necessary to produce an attack. Results of some of these studies are recorded in Fig. 9. It was found that a rest period of seven days resulted in the return of the sensitivity threshold to pollen which existed in the previous experiment. This method was then utilized as the quantitated response to investigate some factors which might affect the threshold.

USE OF THE THRESHOLD PHENOMENON IN SUBSEQUENT EXPERIMENTS

The dog was exposed at weekly intervals to ragweed-pollen-in-water suspension. The pollen was delivered to the chamber for a period of fifteen minutes at the initial concentration; when no reaction occurred at the end of that time, the concentration was doubled and aerosolized for the next fifteen minutes.

This procedure was continued, with the concentration of ragweed pollen doubled every fifteen minutes until definite asthma occurred clinically and as recorded on the kymograph tracing. The results of these studies are shown in Fig. 10. As soon as a definite asthmatic attack occurred, the experiment was stopped and the dog was treated with epinephrine and rewarded with food.

Epinephrine.—Epinephrine shortens the duration of the asthmatic attack of the dog, as described above (Fig. 6). Also, 0.3 ml. of 1:1,000 aqueous epinephrine administered subcutaneously prior to the experiment increased the threshold of concentration of pollen necessary to produce an attack, as shown in Fig. 10.

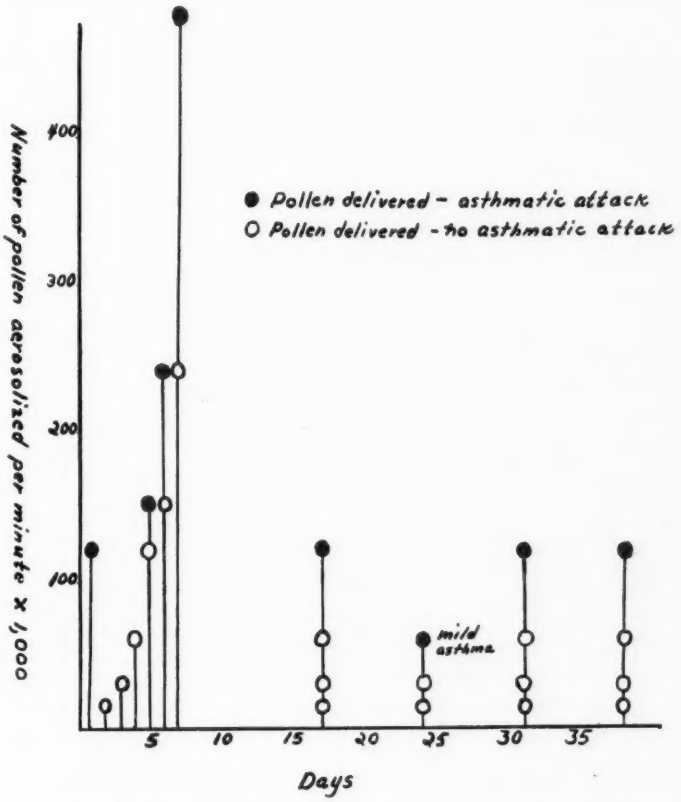

Fig. 9.—Concentrations of pollen required to produce asthma.

Serotonin.—The action of serotonin as an additional humoral mediator in the asthmatic response has been postulated. Serotonin creatinine sulfate, 1.5 per cent, was aerosolized into the animal chamber and no respiratory symptoms were produced in the dog, as illustrated in Fig. 4. This concentration of serotonin was sufficient to produce the transient dyspneic shock syndrome described by many workers in guinea pigs which were placed in the chamber immediately after the dog had been there. A 1.5 per cent solution of serotonin was next used as the suspending medium for ragweed pollen instead of distilled water to

determine whether the addition of serotonin would lower the concentration of pollen necessary to produce an attack. No potentiation of the response with the addition of the aerosolized serotonin was observed.

Lysergic Acid Diethylamide.—The many investigators who have studied its effects have found this agent to be one of the most potent serotonin antagonists. Herxheimer[9] showed that LSD is capable of inhibiting the serotonin dyspneic shock syndrome in guinea pigs, and Fink[10] reported inhibition of the Schultz-Dale reaction in the sensitized mouse by LSD. It appeared of interest to determine the ability of LSD to modify the threshold of concentration of pollen producing the asthmatic response. The dog was given 0.15 mcg. of lysergic

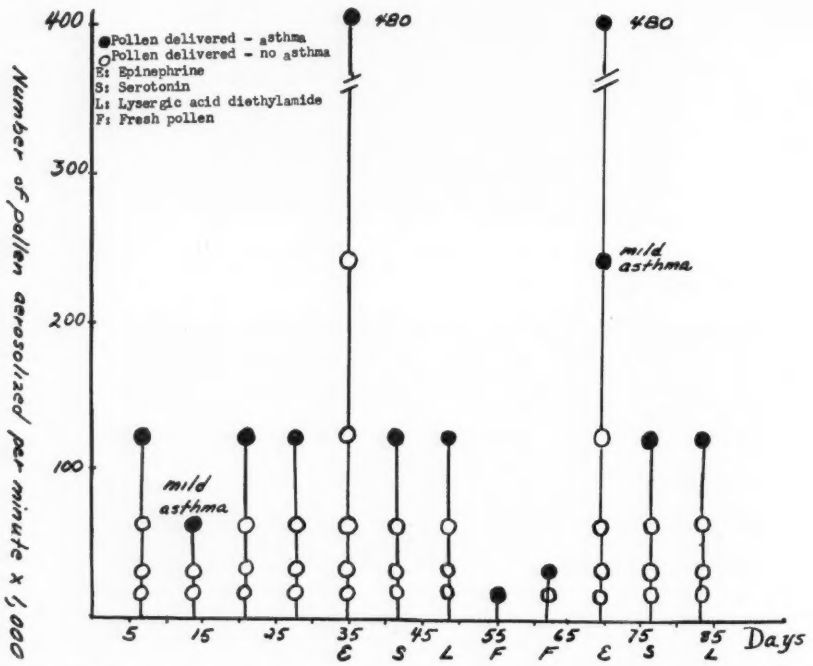

Fig. 10.—Effect of various factors on the pollen concentration required to produce an asthmatic attack in the dog.

acid diethylamide subcutaneously just prior to the beginning of the experiment. This was sufficient to produce definite motor restlessness in the dog but did not modify the asthmatic attack or increase the concentration of pollen necessary to produce the attack.

Fresh Versus Old Pollen.—All previous experiments were conducted with the same supply of commercial pollen obtained two years previously, and this was compared with fresh pollen. Fresh pollen was obtained* out of the ragweed season through a system of forced preseasonal growth and pollination. This pollen was used within two hours of its collection from the plants at 8 A.M., and it was used in the same way as old pollen to produce an attack. The fresh pollen

*From Dr. W. H. Wagner, University of Michigan Botany Department.

showed an increase in antigenic activity over old pollen, as evidenced by the decreased concentrations necessary to produce an attack.

DISCUSSION

It appears evident that the dog described here has ragweed allergy with manifestations suggestive of the response of ragweed-sensitive human beings. It is well recognized that all results and observations depend on the responses of one animal. Confirmation must depend on studies on similar animals, and it is possible that markedly different results may be obtained in other ragweed-sensitive animals when they become available for investigation. We have been told by the veterinary physician supplying the dog that ragweed sensitivity in dogs is not rare in southern Michigan and that these animals may be available for investigation from time to time, depending on their owners. It appears that such animals may prove valuable for certain allergic investigations.

All experiments using the asthmatic response were of short duration, with high doses of pollen being used. The effect of low doses of pollen administered for periods of days must be investigated in the future.

The possibility that the asthmatic response might be conditioned in the dog merely by putting it into the animal chamber without pollen administration after it had had several previous attacks was raised early in the investigation. During the process of working with the recording equipment and evaluating the dog's response, a succession of twenty-five asthmatic attacks were produced in the chamber after exposure to ragweed pollen. Prior to administration of antigen there has been no clinical or kymographic record evidence of any asthmatic symptoms. Environmental and experimental conditions were the same in these experiments. Indeed, since the asthmatic response did not occur below the threshold required in each experiment, it seems evident that the odor of ragweed antigen also did not condition the asthmatic response.

The phenomenon of increasing concentration of ragweed pollen required to produce an asthmatic response when daily experiments were conducted is interesting but not remarkable when such reports as those of Herxheimer[11] and of Swineford and colleagues,[12] who used aerosolized antigen as a means of desensitization in the human allergic patient, are considered.

As described in the clinical history, during the ragweed season the dog had no asthmatic symptoms from atmospheric pollen. It is thought that this is because the threshold of concentration necessary to produce an asthmatic attack is never reached during the ragweed season.

Since it has been reported that symptomatic relief occurs in patients suffering from pollinosis who are exposed to artificial negative ionization of air,[13] experiments are in progress to evaluate this claim, using commercial devices capable of producing negative air ions. Initial results, using the ragweed-sensitive dog in the method described above, indicate that the asthmatic response in the dog to ragweed pollen is not decreased, less severe, or of shorter duration and that the threshold of pollen concentration necessary to produce an attack is not increased by the use of the air-ionizing apparatus. Further results will be presented in a separate communication.

SUMMARY AND CONCLUSIONS

1. A case of spontaneous ragweed hypersensitivity in the dog is described, and clinical and immunologic investigations are reported.

2. The dog with spontaneous hypersensitivity is a suitable model of allergy for experimental work.

3. An asthmatic response in the dog which could be studied in the laboratory was produced by sufficient antigen. Since this response was reproducible and depended on sufficient antigen to produce a response, it may be used as a quantitated response in a rough biologic system.

4. A threshold of pollen concentration was necessary to produce an asthmatic attack in the dog.

5. The threshold of concentration of pollen required to produce an asthmatic attack was increased by frequent asthmatic attacks or pollen exposure and decreased by longer periods of freedom from exposure.

6. Asthma was not conditioned in this dog, in spite of recurrent attacks in the same environment.

7. Serotonin creatinine sulfate, 1.5 per cent, did not produce respiratory symptoms in the dog during aerosolization under the conditions of the experiments described.

8. Aerosolized 1.5 per cent serotonin creatinine sulfate did not potentiate pollen in causing an asthmatic response.

9. Lysergic acid diethylamide did not modify the asthmatic attack or increase the amount of pollen required to produce the attack.

10. A higher concentration of old ragweed pollen than of very fresh pollen was necessary to produce an asthmatic attack.

11. Epinephrine increased the threshold of concentration necessary to produce an asthmatic response.

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SEDIMENTATION STUDIES OF SKIN-SENSITIZING ANTIBODY

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THE skin-sensitizing antibody of human atopic allergy does not readily cross the placental barrier; however, the "blocking" antibody induced by hyposensitization passes the placenta freely.[1] Campbell and associates[2] have demonstrated that Rh saline agglutinating antibody, which also does not cross the placenta readily, is associated with a more rapidly sedimenting fraction of the serum globulins than the Rh incomplete or "blocking" antibody which does pass the placenta. In view of the analogous relationship between the atopic allergy antibody system and the Rh antibody system with regard to placental transfer of antibody, studies were initiated in this laboratory to determine whether skin-sensitizing antibody, like the Rh saline-agglutinating antibody, is also associated with the more rapidly sedimentating globulin fraction.

MATERIALS

Serums were obtained, from twelve allergic persons, which produced strongly positive transfer skin test reactions to various test allergens including grass, weed, epidermal, and mold extracts. The group of patients from whom the serums were obtained included both adults and children suffering from major allergic diseases, such as asthma, hay fever, perennial allergic rhinitis, eczema, urticaria, and various combinations of these. Details of age, sex, diagnosis, treatment history, and test sensitivity are given in Table I. There were approximately equal numbers of serums from treated and untreated persons.

All serums were stored in a refrigerator at 4° C. and processed within six weeks of the time obtained. In the one case in which both pre- and posttreatment sera were obtained from the same patient, the pretreatment serum was stored in the Deep Freeze at –20° C. for six months.

From the Division of Chemistry and Chemical Engineering, California Institute of Technology.

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Skin tests were performed intradermally with 0.02 ml. of 1:500 dilutions of commercial test allergens.*

METHODS

Volumes of 6 ml. of each serum were diluted with an equal volume of 1 per cent saline containing 10 per cent borate buffer (pH 8.4, $\mu = 0.125$) and centrifuged for ten to twelve hours at 40,000 r.p.m. in a Spinco Model L preparative centrifuge. The supernatant was decanted, and the remaining gelatinous pellet of heavy materials was resuspended in the borate saline diluent. Both supernatant and resuspended pellet were recentrifuged through four more cycles at 40,000 r.p.m. for ten to twelve hours. At each cycle any further pellet material

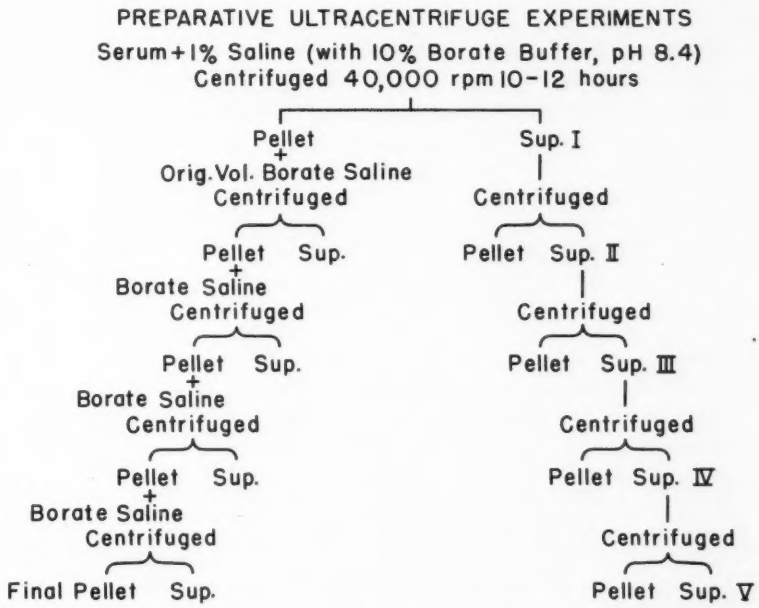


Fig. 1.—Method of serum fractionation by means of preparative ultracentrifugation.

TABLE I. DIAGNOSIS AND TREATMENT STATUS OF THE SERUM DONORS

PATIENT	SEX	AGE (YEARS)	DIAGNOSIS	TEST ALLERGEN	HYPOSENSITIZATION (YEARS)
D. N.	M	22	Allergic rhinitis	*Distichilis spicata*	None
N. N.	M	23	Asthma	*Olea europaea*	None
N. P.	M	24	Hay fever	*Lolium perenne*	None
M. C.	F	15	Asthma and rhinitis	Mixed feathers	3
J. R.	F	10	Asthma and eczema	*Artemesia californica*	1.5
T. V.	M	15	Asthma	Cattle hair	2
J. S.	M	9	Asthma and rhinitis	Altenaria	2
J. B.	M	5	Asthma	*Bromus rigidus*	0.3
M. S.	M	13	Asthma	*Cynodon dactylon*	2.5
A. W.	M	30	Hay fever	*Lolium multiforme*	None
A. L.	M	38	Asthma and urticaria	Rabbit hair	None
P. J.	M	32	Asthma	*Franseria acanthicarpa*	None and 0.5*

*Pre- and posttreatment serums obtained.

*Hollister-Stier Laboratories, Inc., Los Angeles, California.

formed from the supernatants was discarded and the suspending fluid from the pellet materials was likewise discarded. A schematic representation of the fractionation procedure is presented in Fig. 1.

Four serums (Table III), diluted with an equal volume of borate saline, were fractionated by means of a Waugh moving-partition cell[3] in a Spinco Model E analytic ultracentrifuge. The cell was filled with 0.55 to 0.65 ml. of the diluted serum. The runs were carried out at 52,640 r.p.m. at a rotor temperature of 23 to 25° C. The deceleration schedule was started approximately thirty minutes after gaining full speed, when the S19 peak had just reached the initial position of the moving partition. During the course of slow deceleration, the S19 peak moved completely into the bottom compartment. The rotor decelerated from 52,640 to 37,000 r.p.m. on the slow brake, from

TABLE II. PASSIVE TRANSFER TEST DATA ON PREPARATIVE CENTRIFUGE FRACTIONS

PATIENT	SERUM (DILUTED 1:1)	FINAL SUPERNATANT (SUP. V)	FINAL PELLET
D. N.	3+	1+	0*
N. N.	3+	2+	1+*
N. P.	3+	1+	0*
M. C.	4+, 3+	4+, 3+	4+, 3+
J. R.	4+, 3+	3+, 2+	2+, 2+
T. V.	4+, 4+	3+, 2+	3+, 2+
J. S.	4+, 3+	4+, 4+	4+, 3+
J. B.	4+, 4+	3+, 3+	3+, ±
M. S.	4+, 3+	3+, 2+	4+, 3+
A. W.	4+, 4+	4+, 4+	4+, 4+
A. L.	4+, 3+	4+, 1+	4+, 3+
P. J. pretreatment	4+, 3+	3+, 2+	3+, 3+
P. J. posttreatment	4+	4+, 4+	2+, 0

Each score represents an individual test on a sepaarte donor.

*Final pellet diluted to original volume; therefore, protein concentration is 0.1 per cent instead of 0.6 per cent.

TABLE III. PASSIVE TRANSFER TEST DATA ON PARTITION-CELL FRACTIONS

PATIENT	TOP 1	TOP 2	BOTTOM 2	BOTTOM 1*
A. W.	4+, 3+	2+, 2+	3+, 1+	4+, 3+
A. L.	3+, 2+	2+, 2+	1+, 1+	3+, 1+
P. J. pretreatment	4+, 3+	3+, 3+	2+, 2+	3+, 2+
P. J. posttreatment	4+, 2+	2+, ±	3+, ±	4+, 2+

*The fraction "bottom 1" is the only one that contains demonstrable S19 material.

37,000 to 26,000 r.p.m. on the medium brake, and from 26,000 to 6,000 r.p.m. on the rapid brake. After the rotor had reached a speed of 6,000 r.p.m., the final deceleration was carried out on slow brake. The rotor chamber was opened when the speed had been reduced to 1,000 r.p.m., and as soon as the rotor stopped it was immediately removed with utmost gentleness and tipped so as to stabilize the concentration gradient in the partition cell. A syringe and blunt needle were used to remove the fractions from the top and bottom compartments of the cell. In this manner the sera were separated into "top 1" and "bottom 1" fractions. Six such runs were carried out for each serum, and the corresponding "top 1" and "bottom 1" fractions were pooled. A sample of the pooled "top 1" fraction of each serum was examined for the presence of the S19 peak in a regular centrifuge cell at 52,640 r.p.m. in the

Spinco analytic ultracentrifuge. The remaining portions of the "top 1" fractions of each of the four sera were rerun in the Waugh partition cell following the identical time schedule used in the first fractionation. The fractions obtained from the "top 1" fraction by this method were designated "top 2" and "bottom 2."

Sedimentation patterns of the various supernatant and final pellet fractions of the same four sera used for the partition-cell experiments were obtained in the Spinco analytic ultracentrifuge at 52,640 r.p.m. and a rotor temperature of 23 to 25° C. The supernatants were run in the ultracentrifuge undiluted at a protein concentration of 2.5 to 3.5 per cent.

Passive transfer tests were performed with the various fractions obtained by these procedures. Except in the cases of three serums (D. N., N. N., and N. P.), these tests were performed in duplicate with two separate donor subjects for each serum and fractions thereof. The final pellet materials for testing were prepared by resuspension of one pellet, derived from 12 ml. of diluted serum, in 2 ml. of borate saline, giving a protein concentration of approximately 0.6 per cent. Three exceptions to this are indicated in Table II. In the case of these three sera (D. N., N. N., and N. P.) the pellets were diluted to the original volume of diluted serum, so that if all the activity were concentrated in the pellet it could be more easily compared with the skin-sensitizing activity of the diluted serum.

RESULTS

The analytic ultracentrifuge experiments performed with the first supernatants (Sup. I) from the preparative ultracentrifuge fractionations (Fig. 1) were almost completely devoid of the S19 components. The second supernatants (Sup. II) and all succeeding supernatants were absolutely free (within the resolving capabilities of the method) of the S19 components (Fig. 2). Analytical ultracentrifuge studies of the final pellet solution (Fig. 2) showed a high concentration of the S19 components, a smaller S6-7 peak, and a still smaller concentration of much more rapidly sedimenting material (S40-50). The analytical ultracentrifuge experiments carried out with the pooled "top 1" solutions showed absolutely no evidence of any of the S19 components.

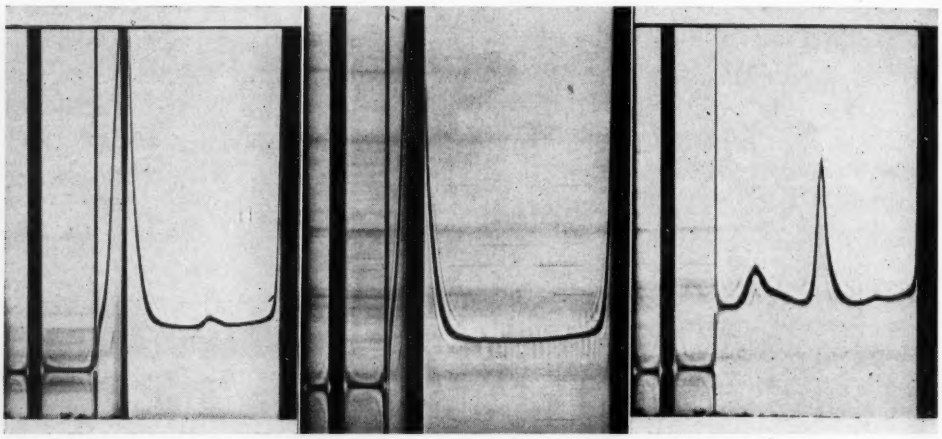
Free boundry electrophoresis experiments carried out with the pellet solutions (0.6 to 1.0 per cent protein in barbital buffer, pH 8.6, $\mu = 0.1$) showed two peaks with mobilities -1.7×10^{-5} and -4×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$. These peaks correspond to fast gamma globulin and alpha$_2$ globulin components. The alpha$_2$ globulin peak constitutes about 55 per cent of the protein present.

Results of the passive transfer skin tests are tabulated in Tables II and III. It is quite evident that skin-sensitizing activity has been neither significantly removed nor significantly concentrated in any of the fractions prepared by either method of fractionation. Control experiments have demonstrated that the borate saline used as a diluent does not influence the passive transfer skin tests.

DISCUSSION

During the preliminary stages of this study, Sehon[4] reported data clearly identifying "blocking" antibody with the S7 globulins. He also drew the inference from his work, using the Waugh partition-cell method and the ragweed allergen system, that skin-sensitizing antibody was associated with the S19 globulins, thus supporting the analogy with the Rh antibody system.

Our data, however, do not support this hypothesis. All our supernatants and "top" fractions containing no detectable S19 peaks show high concentrations of skin-sensitizing activity as compared with whole serum. One cycle of preparative centrifugation was sufficient to reduce the concentration of the S19 components to an almost imperceptible level, and even after five such



A. *B.* *C.*

Fig. 2.—Sedimentation patterns at 52,640 r.p.m. of a serum sample (A. W.) and two of the derived fractions, all in borate saline. *A*, Serum 2 per cent protein concentration thirty minutes after gaining full speed. *B*, Second supernatant (Sup. II), 3 per cent protein concentration, thirty-two minutes after gaining full speed, showing the absence of the S19 components. *C*, Final pellet solution, 0.6 per cent protein concentration, twenty-four minutes after gaining full speed.

successive centrifugations the last supernatant showed skin-sensitizing antibody activity. The final pellet solution contained a high concentration of the S19 material relative to the concentration in serum; however, the pellet solution was no more active than the serum. It is possible that the skin-sensitizing antibody is concentrated in the pellet but is partially inactivated during the process of pellet formation, but this does not seem likely. The pellet solutions containing augmented amounts of S19 material showed almost equal skin-sensitizing activity with both whole serum and the S19-depleted fractions.

Our Waugh partition-cell data are entirely consistent with the data obtained by the preparative ultracentrifuge method. The fractions depleted of S19 material ("top 1," "top 2," and "bottom 2") showed as much antibody activity as fraction "bottom 1" that contained the S19 components. The advantage of the use of the partition cell is that the separations are carried out under direct visual control and under well-defined convection-free conditions.

More recent experiments from Sehon's laboratory[5] have shown that there is no correlation between the concentration of the S6-7 components and the skin-sensitizing antibody activity. It is to be noted that in the methods described, by which our fractions have been obtained, a separation of materials of intermediate sedimentation coefficient selectively into one or another of these fractions was not obtained. The skin-sensitizing antibody may be present in low concentration, even if it is of high activity, in allergic serums: therefore, it is possible that the antibody may be of intermediate sedimentation coefficient and may be divided in approximately equal amounts between the "heavy" and "light" fractions prepared by our methods. It is also possible that the antibody activity could be associated with antibodies of different sedimentation coefficients. If the skin-sensitizing activity were associated with molecules of high molecular weight but low density, this might account for both the lack of placental passage of the antibody and the lack of concentration of the activity in the pellets.

At the inception of this study it was hoped that the skin-sensitizing antibodies might prove to be molecules of the S19 group, thus providing a convenient characteristic for their concentration and purification. The results obtained are thus further disappointing, as they leave the characterization and in vitro manipulation of these antibodies as elusive as they have been for the past decades.

SUMMARY

Serums from allergic patients have been fractionated by preparative and analytic ultracentrifugation into fractions devoid of the S19 components and fractions containing S19 components. Skin-sensitizing activity has been found to be associated with both kinds of fractions. The skin-sensitizing activity could not be correlated with the S19 globulin fraction alone.

It is a pleasure to acknowledge the many helpful suggestions and guidance in ultracentrifuge methods provided by Dr. Jerome Vinograd.

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PRELIMINARY EXPERIMENTS WITH IONIZED AIR IN ASTHMA

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INVESTIGATIONS into the therapeutic potentialities of artificially ionized air were temporarily interrupted by World War II. As a result of Swangard's[1] findings in 1946, however, interest was renewed. It was the reported[2] beneficial effects of air-borne, negative ions on such upper respiratory states as paranasal sinusitis and vasomotor rhinitis that prompted Kornblueh's group[3, 4] to explore their influence in hay fever. Prompt amelioration of symptoms appeared to be the outcome. Currently, this mode of therapy is being recommended for asthma and hay fever through newspaper advertisements by a commercial producer of air ionizers (Puritron).

With these reports in view, we decided to conduct a double-blind experiment in sixteen individuals who were currently in hospital because of chronic asthma.

HISTORICAL BACKGROUND

According to the review of Kornblueh,[3] current information surrounding the use of ionized air stems from the work of Dessauer and associates who used magnesium oxide dust as the carrier of the chosen electric charge. The latters' studies led them to conclude that:

> Fast-moving, small ions were of minor importance, while the slower-moving larger ions tend to be inhaled and partially absorbed by the lungs. Those of intermediate size were the more effective. The ions bearing positive charge moved about 30 per cent slower than negative ions.

Martin[5] had already observed that the concentration of small "ions" decreased as a result of contamination of the air with gases. This was not confirmed by Beckett and Winsor,[6, 7] whose studies were conducted in Los Angeles where "smog" contamination of the air is relatively high. They concluded that small "ions" attach themselves to such air contaminants, thereby increasing the total natural "ion concentration."

Yaglou and his co-workers[8] had observed as early as 1933 that positive ions sometimes caused headaches and irritated the upper respiratory tract, whereas negative ions appeared to confer a sense of well-being. Edstrom,[9] on the other

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hand, felt that some individuals were incapable of responding physiologically to "unipolar air." In general, however, he was of the opinion that air of positive charge tended to exert an influence opposite to that of negative ions.

After World War II, numerous physical and biological experiments were carried out with ionized air, stimulated by the industrial production of ion generators. Krueger and Smith,[10] for example, have recently conducted objective tests on the isolated rabbit trachea and concluded that negatively ionized air enhances ciliary movement and consequent mucus flow. On the other hand, both these functions were inhibited when the opposite polarity was chosen for the experiment. These results appear to support the earlier impression of Yaglou[8] that the clinical effect depended on the sign of the charge carried by the ionized air.

METHODS AND MATERIALS

The purpose of the present experiment was to conduct a double-blind, control study on asthmatic patients during two 120-minute exposure periods to ionized air.

Subjects.—Sixteen chronically asthmatic men and women, ranging in age from fifteen to fifty-three years, were selected for the experiment. With one exception, each had remained asymptomatic for at least three hours beforehand, despite the prohibition of all medication for twelve hours or more.

Procedure.—Immediately before the study, each air-ionization apparatus (Ionaire*) was tested with a Keithly micromicro-ammeter and target collector. As anticipated, it was found that negative or positive ions were being emitted at the desired rate of 1×10^{-10} amperes. In addition, each unit was tested with a Geiger counter and was found to be emanating beta particles at a harmless rate—below that associated with an ordinary wrist watch with a luminous dial. An independent worker then coded each generator after noting whether it was of negative or of positive polarity and placed it in one of eight standard hospital rooms (without air-conditioning). Patients were then assigned to separate rooms by randomization. They sat facing the apparatus at a distance of two feet. At the end of the first two-hour period, the occupant was transferred arbitrarily to another room for an additional two hours of exposure to its coded ionizer. Observations were made on each patient by the physician at fifteen-minute intervals while the patient kept a continuous detailed record of all developments, especially asthma. The intensity of symptoms was designated by the symbols, + for mild, ++ for moderate, and +++ for severe.

As stated above, all subjects, with one exception, had been symptom free for at least three hours prior to the first exposure. In six instances it was necessary to set up a secondary exposure of two hours on the next day, when

*"Ionaire" is the trade name of an ion generator manufactured by Wesix Electric Heater Company of San Francisco, Calif. The active portion is comprised of a tritium foil and a charged plate mounted in a small plastic, protective holder. The tritium emits beta particles of low energy, which in turn create both positive and negative ions. These temporarily affect the charge of the air because ions of one sign are continuously removed by a plate of controlled and opposite charge.

TABLE I. CLINICAL RESPONSES OF TWELVE PATIENTS TO NEGATIVE IONS EMPLOYED IN THE SECOND 120-MINUTE TEST

PATIENT	IONS USED IN FIRST TEST	WHEEZING JUST BEFORE EXPOSURE		RESPONSE TO NEGATIVE IONS									SPUTUM	SIDE EFFECTS IN NOSE AND THROAT
				WHEEZING			DYSPNEA			COUGHING				
		DEGREE	DURATION IN HOURS	DEGREE	ONSET IN HOURS	DURATION IN HOURS	DEGREE	ONSET IN HOURS	DURATION IN HOURS	DEGREE	ONSET IN MINUTES	DURATION IN HOURS		
Mm	Positive	0	¼	+	1¾	¼	+	1	¼	0	0	0	0	0
Ac	Positive	0	2	++	½	¾	+	¼	¼	++	15	1½	0	Clogging
Js	Positive	0	2	0	0	—	—	—	—	0	0	0	0	0
Mg	Positive	0	2	++	¾	1	+	¾	1	+	2	1¾	+	0
Gd	Positive	+++	¾	++	—	2	—	—	—	+	5	2	+	Dryness
Wj	Negative	0	2	+	¼	¼	—	—	—	++	*	1½	+	0
Rt	Negative	++	½	++	—	¼	+	¼	1½	++	*	1½	+	Dryness
Cd	Negative	+	1½	+	—	2	—	—	—	+	*	2	+	Dryness
Ra	Negative	0	2	—	—	—	—	—	—	—	—	—	0	0
Rb	Negative	0	½	+	1	¼	—	—	—	—	—	—	0	Dryness
Pb	Negative	0	2	—	—	—	—	—	—	—	—	—	0	0
Fs	Negative	+	2	+	—	1½	—	—	—	+	*	1½	0	0

*Symptoms present at outset continued unabated.

the polarity to be used was known to the physician. The reason for this was that the original chance assignment had exposed these patients to atmospheric ions of the same sign during all four hours.

RESULTS AND DISCUSSION

Table I presents the detailed results procured in twelve patients during their second two-hour test when negative ions were being employed. For example, M. M. had been exposed during her first two-hour period to positively ionized air. She had maintained a symptom-free base line for a quarter of an hour before the second test. Mild wheezing and dyspnea without cough developed, however, during the fourth hour of testing.

Such data for all forty of the two-hour exposures involved in the total experiment have been condensed in Table II. In other words, the clinical effects of negatively and positively ionized air are summarized for our sixteen patients.

TABLE II. INFLUENCE OF NEGATIVE AND OF POSITIVE IONS ON CLINICAL PATTERNS OF SIXTEEN ASTHMATIC SUBJECTS GIVEN A TOTAL OF FORTY TWO-HOUR EXPOSURES

TYPE OF IONS	TEST NUMBER	NUMBER OF PATIENTS PER TEST	WHEEZING AND DYSPNEA		INTERPRETATION OF RESULTS
			BEFORE TEST	DURING TEST	
Negative	1st or 2nd	8	0	0	No effect
Negative	3rd	1	0	0	No effect
Positive	1st or 2nd	13	0	0	No effect
Positive	3rd	6	0	0	No effect
Negative	1st or 2nd	5	+ or ++	+ or ++	No effect
Positive	1st or 2nd	1	+ or ++	+ or ++	No effect
Positive	3rd	1	+ or ++	+ or ++	No effect
Negative	1st or 2nd	3	0	+	Developed slight wheezing
Positive	1st or 2nd	1	0	+	Developed slight wheezing
Positive	1st or 2nd	1	0	++	Developed moderate wheezing

In twenty-eight instances, it will be noted, the patients started the exposure period with a symptom-free base line. This clinical pattern was unchanged by a two-hour exposure to either positive or negative ions. In the case of seven other tests, slight or moderate wheezing was already present just before exposure, and symptoms continued unaltered throughout the 120-minute period, regardless of the charge of the ion. Thus, thirty-five of forty exposures failed to effect any change in the clinical pattern for better or for worse.

In the five remaining tests, three of which employed negative ionization while two involved the opposite polarity, it appeared that mild or moderate wheezing and dyspnea had occurred where none had previously existed. This might, of course, have been the situation had no treatment been given. Indeed, this assumption seems justified by the results just noted for the thirty-five exposures, none of which exerted any influence on the patient's pattern of clinical behavior.

SUMMARY

A two-hour exposure of fifteen symptom-free patients to artificially ionized air of concealed polarity, followed by a repeat experiment with the opposite charge, revealed no difference in the biologic effect of positive and of negative atmospheric ions. To our surprise, the negative (like the positive) ions did not appear to influence the patient's typical pattern of wheezing and of remission. Dryness of the nose or throat was also a frequent side effect, regardless of charge.

Contrary to expectations, wheezing in symptom-free asthmatic subjects seemed to occur with negative ionization of the air in some instances. No amelioration of this symptom once present was noticed when subjects were similarly exposed. Being essentially a double-blind study, it is unlikely that psychogenic influences colored these results.

These findings discouraged us from further pursuit of the alleged therapeutic benefits of negative ionization in asthmatic patients.

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THE PERIOD OF SENSITIZATION IN IMMIGRANT HAY FEVER PATIENTS

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DURING the ragweed pollen season of 1959, we became cognizant of the number of immigrant patients whom we were treating for hay fever. This aroused our interest in trying to ascertain the number of ragweed pollen season exposures required for these persons to become sensitized. This interval of time shall be referred to as the "period of sensitization."

We studied a series of hay fever cases involving immigrant patients. These cases were collected from the following sources: (1) our private practice, (2) resident physicians in various hospitals in the Cleveland area, (3) the Mt. Sinai Allergy Clinic, and (4) one case from a colleague in the practice of allergy. We interviewed all but one of the patients personally and skin-tested all but three of the patients included in the study. These three had been skin-tested by other allergists, who reported positive skin test reactions to ragweed pollen extract. These patients likewise gave clear-cut histories of ragweed pollinosis.

This group of thirty-two immigrants consisted of fourteen female patients and eighteen male patients. Their age distribution is recorded in Table I.

TABLE I. AGE RANGE OF PATIENTS

AGE (YEARS)	NUMBER OF CASES
10-20	2
21-25	3
26-30	13
31-35	6
36-40	4
41-45	1
46-50	1
51-70	2

In interviewing these foreign-born subjects, we were particularly interested in determining the month and year during which they came to the United States, where they lived, and in what year their hay fever symptoms began. We were also interested in whether any of these patients had had allergic difficulty prior to coming to the United States and whether there was any family history of allergic disease among their brothers, sisters, parents, grandparents, aunts, and uncles.

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Table II shows a breakdown of the group's geographic distribution.

TABLE II. GEOGRAPHIC DISTRIBUTION

COUNTRY OF BIRTH	NUMBER OF CASES
Germany	3
Czechoslovakia	2
Italy	5
Turkey	3
Spain	1
Hungary	1
Lithuania	1
Poland	4
Scotland	1
Yugoslavia	1
Philippines	5
China	1
Puerto Rico	2
Mexico	2

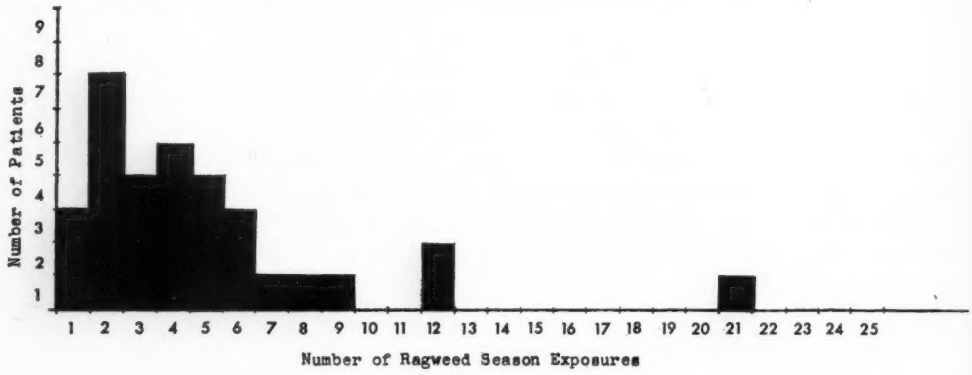

Fig. 1.

The "period of sensitization," as we shall define it, is the number of ragweed pollen seasons to which a patient has been known to be exposed. We decided to arbitrarily define the ragweed pollen season in the Cleveland area as the period from August 15 to October 1. In our tabulation a person exposed to ragweed pollen, even to as little as one day's exposure, was considered to have been exposed to one ragweed season. Our criterion for the clinical diagnosis of ragweed hay fever consisted of a positive seasonal, clinical history. This was corroborated by a positive immediate type of skin wheal response to ragweed pollen extract. We limited our choice of cases to clear-cut cases of pollinosis that met the requirements just described.

The results of this study are shown graphically in Fig. 1. As can be readily noted, the majority of the immigrant patients developed ragweed pollinosis after a period of sensitization of six or less ragweed season exposures. The average exposure time was 4.9 seasons, while the mean exposure time was 4.0 seasons. The mean refers to the midpoint of the series below and above which there is an equal number of cases.

Several examples of the problem that can be encountered in such a survey will be presented briefly.

Patient S. B., a 29-year-old man, arrived in the United States in December, 1947, and settled in Cleveland, Ohio. He moved to California in November, 1949, and returned to Cleveland in September, 1952. It was therefore felt that the summers of 1950 and 1951 need not be included in determining the length of his exposure to giant and dwarf ragweed pollen. He first developed symptoms in 1955 and was considered to have been exposed to five ragweed seasons.

Patient F. T., a 24-year-old woman, originally came to the United States in September, 1956; she visited Cleveland for two days and Florida for approximately two weeks. She returned to Puerto Rico and then re-entered the United States in May, 1957. The short exposure time in September, 1956, was considered as one exposure year. Patient F. T. experienced hay fever in August, 1958, and therefore had two prior season exposures.

Two resident physicians gave histories of having developed ragweed pollinosis during their first exposure to ragweed pollen. Patient R. G. was a 29-year-old Mexican who was born and lived in Mexico City. His past history revealed that he had bronchial asthma during his youth. He came to the United States for the first time in June, 1956, and in August and September of that year he began having paroxysms of sneezing and rhinorrhea. Besides a positive test reaction to ragweed pollen extract, he gave a negative test reaction to 1:40,000 Endo dust and a questionably positive reaction to grass and tree pollen extracts. The other resident physician, Patient J. C., a 28-year-old Filipino man entered the United States on Oct. 24, 1954. He began having rhinorrhea, lacrimation and itching of the eyes, nasal stuffiness, and occasional bouts of asthma in August, 1955. This patient had a strong family history of allergic difficulty, and prior to coming to the United States he occasionally noticed itching of his eyes on a perennial basis. Each of these last two cases was arbitrarily given a period of exposure of at least one ragweed pollen season.

Table III presents the results concerning the patients' personal past histories and their family histories of allergy. All persons in the study were questioned, and those admitting to family or personal history of allergy were recorded as having given positive replies.

TABLE III.

TOTAL CASES	POSITIVE REPLIES	PER CENT
	Personal Past History of Allergy	
32	6	19
	Family History of Allergy	
32	9	29

We were further interested in determining the period of sensitivity in patients who had had previous allergic difficulty as opposed to the group whose initial symptomatology began in the United States. In the group of six patients with a positive past history of allergy, the average number of season exposures was 2.5, as opposed to 5.4 in the twenty-six patients without

prior difficulty. Likewise, in the nine cases in which there was a positive family history of allergy, the average period of sensitivity was 3.5 versus 5.4 in the group with negative family history. Combining all cases with personal and/or family histories of allergy, we find that there was a group of eleven patients whose average length of prior exposure was 3.0 ragweed seasons. In the remaining twenty-one cases, the average time was 5.8 seasons. In this series of analyzed cases, it certainly appears that the group with personal and family histories of allergy developed ragweed pollinosis in a shorter period of time than the rest of the foreign-born patients.

DISCUSSION

A search of the literature revealed only two articles since 1940 in which the authors attempted to deal with this problem. In 1953 Shilkret[1] reported a study of 162 immigrant hotel employees who had developed hay fever since residing in the United States. His figures reveal that this group of patients required, on the average, 15.8 years of exposure before they developed ragweed pollinosis. In a more recent article, Hughes[2] discusses this problem in recent immigrants to Canada. Of the sixty cases in his series, two patients developed hay fever in the first year of residence in Canada, four in the second year, thirteen in the third year, thirteen in the fourth year, and the remaining twenty-eight developed symptoms between the fifth and fifteenth years of residence in Canada.

The results of our study seem to agree more closely with the data presented by Hughes. There are many variables that certainly would have an important bearing on such a survey. These include the quantitative pollen counts[3] in areas where the immigrants have resided and the age distribution of the patients in a particular study.

Theoretically, these immigrants had never been exposed to ragweed pollen prior to coming to the United States. After exposure to this material over a varying number of seasons, they developed clinical allergic sensitivity with manifest symptoms of hay fever.

Since one of our assumptions is that these persons have never been exposed to and therefore sensitized to ragweed pollen, it is of interest to review the world-wide distribution of ragweed pollen as described by various authorities.[4-7]

Ambrosia elatior (short ragweed) and *Ambrosia trifida* (giant ragweed) grow abundantly on the North American continent, where its limits are described as south of latitude 50°, north of latitude 25 to 30°, and east of the Rocky Mountains. North of latitude 50°, in such areas as Alaska, Northern Canada, and Scandinavia, the ragweed plant does not thrive. Atmospheric pollen counts in the western states do reveal occasional giant and dwarf ragweed pollens, but usually in "subclinical" amounts. The northern border of Mexico, adjacent to the United States, experiences moderate elevations of ragweed pollen which can cause clinical symptoms. In Mexico City, Cuba, Puerto Rico, and Central America ragweed flora is insignificant. Outside the continental

United States, the presence of ambrosia and related flora has been reported from time to time in such areas as the British Isles, France, Italy, and many other countries of Europe, Asia, and Africa. However, the cases of true ragweed pollinosis reported from these areas have been infrequent indeed. The occurrence of ragweed pollen in other countries, although in clinically insignificant amounts, may be all that is required to sensitize a person with an allergic predisposition. This may explain why some immigrants experience symptoms during their first exposure to ragweed pollen in the United States. In South America, between southern latitudes 30 and 50°, *Ambrosia tennifolia* and *A. artemisiaefolia* flourish and produce a clinically significant ragweed season during the months of January, February and March.

We interviewed nine foreign-born resident physicians at Mt. Sinai Hospital in Cleveland. None of these nine persons complained about symptoms of ragweed hay fever, and none gave a history of past difficulty with ragweed pollen. Their periods of residence in the United States ranged from three months to thirty-nine months. Four physicians from the Philippines, one from the Dominican Republic, and one from Belgium had negative skin tests to trees, grass, and ragweed pollen extracts. One physician from Argentina, who has lived in Cleveland since June, 1956, gave a good 2 plus reaction to ragweed pollen. Another resident physician from Argentina, with a personal past history of asthma and perennial nasal stuffiness, remarked that his nasal symptoms had subsided since his coming to the United States in June, 1959. He was still experiencing slight itching of the eyes upon arising in the morning. He gave a 2 plus positive reaction to house dust, feathers, and grass, a 1 plus positive reaction to ragweed and alternaria, and a negative reaction to tree pollen mixture. A 24-year-old Mexican physician with no complaints also gave positive skin test reactions to grass and ragweed pollen extracts.

It is interesting to note that our figure of a 29 per cent familial incidence of allergic difficulty, as shown in Table III, compares closely with the figure of 22.5 per cent in Shilkret's series.

SUMMARY

Thirty-two immigrant patients were studied in an effort to determine the length of time that it takes for them to develop ragweed hay fever. The data show that the average length of time before clinical sensitivity appears is 4.9 season exposures. The mean figure for this group is four season exposures.

Nine foreign-born resident physicians were interviewed. None of these gave a history of ragweed hay fever, but three of the nine had positive skin test reactions to ragweed pollen extract. It would be interesting to see whether or not these three eventually develop overt ragweed pollinosis.

The group of patients with personal and family histories of allergy developed ragweed hay fever symptoms in 3.0 season exposures, as compared to 5.8 season exposures for the remainder of the group.

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CORRESPONDENCE

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TO THE EDITOR:

In the introduction of our paper, Purification of Antibodies. I. Dextranase Purification of Antibody Precipitated from Type II Antipneumococcal Rabbit Serum by Dextran, which appeared in this JOURNAL, 30: 433, 1959, the statement was made: "This is believed to be the first demonstration of the successful purification of precipitated antibody by enzymatic elution of the precipitating hapten."

Dr. E. A. Kabat was kind enough to call our attention to the elution of precipitin from a specific mucopolysaccharide-anti *M. lysodeikticus* precipitate by Feiner, Meyer, and Steinberg (Bacterial Lysis by Lysozyme, J. Bact. 52: 375, 1946) and to an abstract of his (Dr. Kabat's) report in Science, 120: 782, 1954, in which he eluted human antidextran precipitins from dextran-human antidextran precipitate.

I am grateful to Dr. Kabat for calling my attention to these prior examples of enzymatic elution of precipitating haptens.

A copy of this letter will be included in any future reprints of our paper.

OSCAR SWINEFORD, JR., M.D.

March 4, 1960

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504 Broadway
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TO THE EDITOR:

We, who have been pondering about the role of the eosinophilic leukocyte in the allergic reaction, may learn some interesting information from the recent experimental studies of Welch and Greer.* Briefly, their study was conducted as follows: In a group of rats, Compound 48/80 (histamine liberator) in Tyrode's solution was injected into the intraperitoneal cavity. In a period of 15 minutes fluid was aspirated from the peritoneal cavity, smears were made and stained and then examined by electron microscopy. The peritoneal cavity of the rat is an ideal location for this type of study since a rich harvest of mast cells, eosinophils, histiocytes, lymphocytes, and mesothelial cells can be obtained normally from this region.

Previous work by Bloom, Larsson, and Smith** showed that the administration of Compound 48/80 produced mast cell degranulation; and now Welch and Greer have shown that these granules are phagocytized by the eosinophils. The electron microscopy studies revealed that the granules, identical to those in mast cells, are incorporated within the cytoplasm of the eosinophils. Their explanation is that mast cell granules are known to be a potent source of histamine, and histamine alone produces an increased number of eosinophils in the circulating blood. A chemical substance with antihistamine properties has been obtained from eosinophils, and it has been postulated that the eosinophilic leukocyte acts as an inactivator of histamine and histamine-like substances. The fact that this cell engulfs mast cell granules may indicate a common line of defense. Thus, in the event of marked histamine release, the eosinophil could serve as a means of rapid isolation and neutralization of the histamine laden particles. "Basophilic bodies" in the cytoplasm of human eosinophilic leukocytes have been described by many investigators. It has been suggested by Di Mayorca*** and his group that the phagocytosis of mast cell granules may be the basis for the "basophilic bodies" seen in circulating eosinophils in human subjects.

SIMON S. RUBIN, M.D.

March 31, 1960

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*Phagocytosis of Mast Cell Granule by the Eosinophilic Granule in the Rat, Am. J. Path. 35: 103-111, 1959.

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***Rendic. Ist. lombardo Sc. 90: 559-572, 1956.

Oil Emulsions in Allergy: A Critique

TO THE EDITOR:

The increasing interest in the use of antigens emulsified in oil, especially the single injection modification, merits consideration of certain aspects of this matter which have not been adequately discussed. In 1934, I became interested in the problem of increasing the safety and effectiveness of pollen desensitization by slowing the absorption of injected extracts, thus permitting the use of large doses and reducing the number of injections required. The use of vegetable oil with lanolin as the emulsifying agent had been described for drugs and diphtheria toxoid, and seemed a good technique for use with other antigens. The effectiveness of this method was studied by emulsifying phenolsulfonphthalein and measuring the urinary excretion after injection into normal people, and then by injecting emulsions of Pitressin emulsified in oil into patients with diabetes insipidus. The first method showed a low even level of absorption and excretion and the second showed a prolonged action of Pitressin as compared with Pitressin solutions. Pollen extract emulsions were then tried in sensitive patients and a series treated in 1937 was reported a year later in the New England Journal of Medicine. I used this method for several years thereafter attempting to improve the manufacture and clinical application of this technique. Mineral oil which could not be metabolized and was considered possibly carcinogenic was avoided. The clinical desensitization was very effective, with a reduction in the number of injections usually required. However, this method of delaying absorption by physical means did not seem a safe practical one for commercial manufacture and distribution, since the emulsions might separate to some degree in the year or two from manufacture to ultimate injection, and deterioration of the antigen would occur since glycerinated extracts could not be used. Careful consideration of dosage was necessary to avoid excessive reactions. I therefore sought increased safety by chemical modification of the antigen and used tannic acid precipitates suspended in oil in various modifications and, finally, since 1953, formalinized pollen tannate suspensions in water.

About ten years after my studies with oil emulsions were begun, Dr. Mary Loveless reported her first experiments with vegetable oil emulsions and later her use of mineral oil. Finally she advocated a one injection technique for pollen desensitization, using an eye test to determine the patient's maximum tolerance. More recently Dr. Ethan Allen Brown of Boston has reported the use in a large number of patients of the Loveless "one shot" mineral oil emulsion given in multiple injections at short intervals at one visit after premedication with antianaphylactic drugs. This has stimulated a wide active interest in this technique among allergists. Because of my long experience with this general problem, I feel obligated to raise certain questions which require careful thought.

The injection of oil introduces a complicating factor of granulomatous reactions which are well known to pathologists. They probably occur more commonly with mineral oil than vegetable oil, as seen in the case of lipoid pneumonia. Tissue necrosis with "cold abscess" formation is apt to develop in a certain proportion of cases, possibly more so with mineral oil than with vegetable oil. Such "cysts" have already been reported by Brown. Vegetable oils for injection into man have been widely used for many years. Although animal studies with radioactive material bear out Dr. Loveless' clinical impression that mineral oil emulsions are more effective than vegetable oil emulsions in prolonging absorption, the question arises whether the difference is worth the additional risk.

The proponents of the mineral oil emulsions have not reported any studies of the absorption rate in either animals or man. There is available the study of Talmage and Dixon using radioactive animal protein emulsified in mineral oil which shows an absorption rate of 20 to 30 per cent in the first twenty-four hours after injection, with a half-life of fourteen days thereafter. My own unpublished studies with mineral oil emulsions of radioactive ragweed, injected subcutaneously into guinea pigs, showed a half-life of about six and one-half days. Allowing a half-life of seven days and disregarding the factor of deterioration of the retained allergen, after injection of 5,000 units of pollen extract emulsified in mineral

oil there would be about 200 units of pollen left after four weeks, which means 100 units would be released the following week, averaging about 15 units a day. After eight weeks about 10 units would be present, with a daily release of less than one unit. If, as is likely, the initial large quantities absorbed had a desensitizing effect, it is hard to believe the subsequent small quantities could have a significant desensitizing effect. On the other hand, if it is believed that small doses do have a worth-while effect, there is no need for assuming the risk of the initial high dose. Furthermore, it would seem reasonable that greater desensitization would result from a more conventional schedule of dosage starting with a dose which the patient could tolerate safely, allowing a week or more for desensitizing effect and increasing each subsequent dose as the tolerance increased. This would result in a larger dosage than the "one shot" method and, presumably, give better clinical results with greater safety. Although good results are reported with the "one shot" method, careful evaluation of results with both methods should be made before advocating the "one shot" for general use simply for the sake of expediency.

In considering improvements in the desensitization method of treatment, better clinical results are important, but the first consideration should be the factor of safety. The "one shot" oil emulsion has been said by Dr. Loveless to produce fewer general reactions than the conventional method and to be, therefore, safer; but Dr. Brown has stated the method is "not for the faint-hearted" physician to use, and that it requires elaborate premedication and resuscitative equipment. The method depends on giving the patient as large a dose as he can tolerate. This tolerance is determined by eye testing, with or without skin testing. It does not allow for wide individual variations, nor can it measure factors which influence the tendency to general reactions which are not directly related to the allergy. It is inevitable, especially so with new patients, that in determining the maximum tolerance dose, the dose will be underestimated in some and, more important, overestimated in others. Experience in allergy should teach the need for caution in estimating the maximum tolerable dose and the obligation to the patient to err on the side of safety. Also the possibility of technical error and the element of human fallibility is ever present. The hazard involved in the "one shot" method is recognized by its proponents in the need for premedication with antihistamines and epinephrine in the form of Susphrine. Such measures are effective in slight reactions. They should not be expected to prevent a severe or delayed anaphylactic reaction. In fact they may give a false sense of security and lead to risking greater dosage.

To estimate and inject the maximum tolerable dose in the case of a sensitive guinea pig is one thing; in the case of a patient it becomes a totally different matter. Although the field of allergy lends itself to experimentation, there are certain areas which in principle may be considered inapplicable to man. For example, it would be generally agreed that it is unsafe to inject intravenously a specific antigen into hypersensitive people. Likewise the idea of injecting into man a maximum tolerance dose subcutaneously should be considered unsafe in principle, since there is no way of being certain of the maximum tolerance dose, and the result of an overdose may be the same as with an intravenous injection. When necessary, calculated risks are taken deliberately in medical practice. Does the treatment of a benign condition such as hay fever require such risk-taking?

To sum up, the increased interest in the problem of improving the methods of clinical desensitization is warranted. Although I believe oil emulsions in increasing dosage will produce greater desensitization with fewer injections than the conventional allergen solutions in water, their use presents important practical problems. Other methods should be actively investigated. The concept of the one injection desensitization treatment with the maximum dose of allergen emulsified in oil as currently advocated is, I am led to believe, inherently wrong from the standpoint of effectiveness and safety when applied to man and should not be recommended at this time as the method of choice for general clinical use.

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OBITUARIES

ARTHUR FERNANDEZ COCA

Arthur Fernandez Coca died in Ridgewood, N. J., on Dec. 12, 1959, at the age of eighty-four years.

Dr. Coca was born in Philadelphia, Pa., on March 20, 1875. He was granted a Bachelor of Arts degree in 1896 and a Master of Arts degree in 1899 by Haverford College. His M.D. degree was obtained from the University of Pennsylvania Medical School in 1900. He spent two years in graduate work at the University of Heidelberg, Germany, followed by two years in bacteriology in the Bureau of Science, Manila, Philippine Islands. He taught bacteriology and immunology in the Cornell University Medical School from 1910, retiring as Professor of Immunology in 1932. He also had been Clinical Professor of Medicine in the Post-Graduate Medical School, Columbia University. He became Medical Director of Lederle Laboratories in 1931, retiring in 1948.

Dr. Coca was founder of the Journal of Immunology in 1916 and was its Editor-in-Chief for thirty-two years. He was also an early member of the Society for the Study of Asthma and Allied Conditions and served as its President in 1931. He was on the Editorial Board of the Journal of Allergy for many years.

He was a member of the American Association of Immunologists from 1916, serving for twenty-two years as its secretary-treasurer, and for the last eleven years of his life as its Honorary President. His early interest in allergy was shown by his being the principal author of "Asthma and Hay Fever in Theory and Practice" by Coca, Walzer, and Thommen, published in 1931 by Charles C Thomas. This volume served as a standard textbook on allergy for many years, both in the United States and abroad. His other publications included four other books and over one hundred and eighty articles in scientific and medical periodicals. He was a member of many scientific and medical societies.

Dr. Coca's contributions to the field of allergy were numerous and important. Some of his later views were not generally accepted, although their originality and his integrity in presenting them were recognized.

Dr. Coca is survived by his widow and by one son and one daughter.

Howard Osgood.

KENNETH E. CROUNSE

KENNETH E. CROUNSE of Delray Beach, Florida, died December 24, 1958. Dr. Crounse was born in Albany, New York, in 1897. He graduated cum laude from the Albany Academy in 1916, after which he served in World War I. In 1920 he graduated from Yale University and in 1924 he received a degree in medicine from Albany Medical College. Dr. Crounse was honored by election to both Phi Beta Kappa and Alpha Omega Alpha.

Dr. Crounse was associated in the practice of internal medicine with Dr. L. Whittington Gorham before specializing in allergy. He spent many years teaching this specialty as Associate Professor of Medicine in Albany Medical College. Ill health forced his retirement in 1956.

Dr. Crounse is survived by his widow and two sons, Dr. Kenneth L. Crounse of Oak Ridge, Tennessee, and Dr. Robert G. Crounse of the National Institutes of Health.

Homer E. Prince

BERNARD GELFAND EFRON

BERNARD GELFAND EFRON died Feb. 16, 1960, in New Orleans, of a cerebral vascular accident after a prolonged and incapacitating illness.

Born Nov. 22, 1902, he received his formal education in the New York State Public Schools, Mt. Vernon College, The University of Maryland, and Tulane University School of Medicine.

Dr. Efron was elected to membership in The American Association for the Study of Allergy in 1932 and was advanced to fellowship in 1935, becoming Fellow of the Academy on amalgamation of the two predecessor societies. He served on the Research Council of the Academy at different times as an active member of its subcommittees on Dust, on Testing Techniques, and on Miscellaneous Inhalants. He became an Emeritus Fellow in 1956.

His astuteness was manifest early in life. Having received no formal high school education, he educated himself in high school subjects and passed examinations which enabled him to enter college. He repeated this process later in life when he discovered that statistical analysis was necessary to assess properly the data from his experiments. He studied biostatistics and became a recognized expert in this field.

He will be remembered best for a method of purification of house dust extract (Boatner, C. H., Efron, B. G., and Dorfman, R. I.: Preparation of Purified House Dust Extracts, Science 91: 389, 1940). However, one of his major contributions was a study revealing the unitarian and specific nature of this antigen by comparative skin tests with extracts prepared from multiple sources. He also was one of the first to employ the statistical method for determining the diagnostic efficiency of extracts.

Excellent monographs on these subjects were published, "The House Dust Factor" *in* Treatment of Bronchial Asthma, edited by Derbes and Engelhardt, and for The International Correspondence Club of Allergy, "Biologic Analysis in Allergy."

Bernie Efron was an indefatigable worker. He was always to be found nights, Sundays, and holidays working in his laboratory. He kept detailed notes of all his experiments. His arguments were poignant, lucid, and backed by scientific analysis. Allergy lost one of its keenest and most colorful students in Bernie Efron.

He is survived by his widow, a daughter, two brothers, and a sister.

Stanley Cohen.